

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Ngai et al.

Group Art Unit: 1655

Serial No. 09/597,608

Examiner: Taylor, J.

Filed: June 20, 2000

Attorney Docket No. B00-100-1

For: Normalizing and Amplifying RNA

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CERTIFICATE OF TRANSMISSION
I hereby certify that this communication is being transmitted by facsimile to the
Comm for Patents at (703) 305-3014 on March 15, 2001.

Signature

Richard Aron Osman

RESPONSE

The Commissioner for Patents
Washington, DC 20231

Dear Examiner Taylor:

Thank you for the Office Action dated Feb. 26, 2001.

35USC131

The present Office Action is the second, consecutive non-final examination on the merits of this application. This Action exceeds the Commissioner's authority to subject Applicants to "an examination" (not multiple, piecemeal examinations) under 35USC131. See also, 37CFR1.104(b) which provides that an Examiner's Action "will be complete as to all matters." On this basis, we submit that the Commissioner has exhausted statutory authority to further reject this application, and on this basis alone we request immediate allowance of the application.

35USC102 and 35USC103

For more than twenty-five years, the CCPA and the Federal Circuit have pointedly expressed frustration with Examiners who persist in making unlawful and illogical rejections based on some notion that printed matter limitations may be ignored. Incredibly, this practice continues.

Claims 19 and 20 are in compliance with 35USC102 and 35USC103. The pending art

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FIG. 1 illustrates the structure and composition of enamel through a perspective view (i.e., FIG. 1A) and a plan view (i.e., FIG. 1B), respectively, of a section of enamel tissue; and

FIG. 2 illustrates a cross-section of a tooth clarifying the structure and composition of dentin.

EXAMPLE 1

Preparation of Etching Compositions

Aqueous solutions of citric acid at saturation, pH about 1, ortho-phosphoric acid, at a concentration of 37% by weight, pH about 1, and EDTA at a concentration of 25%, pH about 7, are prepared for use in the experiments.

EXAMPLE 2

Preparation of an EDTA-Composition

An aqueous composition is prepared by dissolving about 25% of EDTA in water using NaOH as a pH-controlling agent to give a pH about 7. To the resulting solution sodiumcarboxymethylcellulose is added to form a gel-like viscous composition which is easy to handle in the conditioning procedure and does not flow away from the area to be treated. For obtaining a suitable viscosity a quantity of sodiumcarboxymethylcellulose within the range about 3 to about 5% is suitable.

EXAMPLE 3

Preparation of a Conventional Etching Composition

An aqueous solution of ortho-phosphoric acid having an acid concentration of about 37% is prepared resulting in a pH of the solution of about 1. The resulting solution is made viscous in the same manner as described in Example 2 above.

EXAMPLE 4

Extracted human teeth having dental cavities with exposed dentin and enamel are immersed into the aqueous solutions prepared according to Example 1 above. The teeth are held in the solutions for more than 10 min and are then prepared for examination.

All the solutions remove smear and debris within a short period of time (less than 1 min). Conventional acid etching using citric or phosphoric acid produce an essentially smooth dentin surface with only occasional morpuous deposits in the area between the dentinal tubules, but no fibers are visible. Dentinal tubules are clearly visible and appear widened. Etching using EDTA produces a completely different texture with the dentin in-between dentinal tubules consistently displaying a fibrous mesh-work with individual fibers clearly visible and comparable in size to collagenous fibers. Conventional acid etching erodes enamel rods already after 5 to 20 seconds uncovering protruding rod sheaths. EDTA produces similar result only upon exposure for more than 10 min.

The results from the experiments performed show that the collagenous matrix is left intact following EDTA etching, while etching with conventional etching agents, such as citric or phosphoric acid, will dissolve both the mineral and the collagenous matrix of dentin. Etching using EDTA of cavities in preparation for bonding of resin-based fillings are therefore preferred over etching using citric or phosphoric acid. Etching of enamel with EDTA is, however, clinically

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impractical due to the need for exposure times in excess of 10 min. Therefore, enamel is preferably etched with a conventional etching acid, such as citric or phosphoric acid, the etching taking less than 20 seconds for obtaining the desired result. Thus, the invention is based on the concept of using different etching compositions for selective use on dentin and enamel, the compositions being based on EDTA and a conventional etching acid, respectively. For practical purposes the etching compositions are preferably in a viscous form to allow full control of application and to avoid accidental etching of dentin with conventional etching acid, such as phosphoric acid. It is to be noted that etching the dentin part of the dental cavity using EDTA need not be restricted to the dentin part only, since the EDTA composition does not deleteriously affect the enamel part of the cavity. However, care should be taken that the conventional etching acid composition is excluded from coming into contact with the dentin part of the dental cavity.

What is claimed is:

1. A kit for use in the conditioning of dental cavities by etching in preparation for bonding restorations to enamel and dentin, comprising the following items:

- a first container holding an aqueous composition containing EDTA in a concentration which is no less than about 90% of the concentration at saturation of said "acid" has been changed to EDTA;
- a second container holding an aqueous composition containing an etching acid selected from the group consisting of phosphoric acid and citric acid; and
- instructions for the use of the kit by etching the dentin part of a dental cavity using said aqueous composition containing EDTA, and by etching the enamel part of said cavity using said aqueous compositions containing said etching acid.

2. A kit according to claim 1, wherein said acid of said second container b) has a pH of about 1.

3. A kit according to claim 2, wherein said acid of said second container b) is phosphoric acid present in a concentration not exceeding about 40% by weight.

4. A kit according to claim 3, wherein the composition of the first container contains, based on the water contents of the composition:

EDTA in an amount of about 22 to 27% by weight;
sodium hydroxide as a pH-controlling agent in an amount resulting in a pH within the range about 6.5 to about 7.5; and

carboxymethyl cellulose (CMC) or a salt thereof as a viscosity-increasing agent in an amount of from about 1% by weight to about 5% by weight.

5. A kit according to claim 1, wherein the composition of the first container a) contains, based on the water contents of the composition:

EDTA in an amount of about 22 to 27% by weight;
sodium hydroxide as a pH-controlling agent in an amount resulting in a pH within the range about 6.5 to about 7.5; and

carboxymethyl cellulose (CMC) or a salt thereof as a viscosity-increasing agent in an amount of from about 1% by weight to about 5% by weight.

6. A kit according to claim 5, wherein the respect to the composition of the first container a):

the amount of EDTA is about 25% by weight;
the pH of the composition is around neutral pH 7; and
the viscosity-increasing agent is sodium carboxymethyl cellulose in an amount of about 3 to 5% by weight.

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Asp Arg Val Tyr Ile His Pro Ile
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We claim:

1. A method for increasing blood flow to ischemic cardiac tissue comprising the administration of an amount effective for increasing blood flow to ischemic cardiac tissue of at least one active agent comprising a sequence selected from the group consisting of angiotensinogen, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, and SEQ ID NO:42.

2. A kit for increasing blood flow to ischemic cardiac tissue, comprising:

(a) an amount effective for increasing blood flow to ischemic cardiac tissue of at least one active agent comprising a sequence selected from the group consisting of angiotensinogen, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID

NO:13, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, and SEQ ID NO:42 and;

(b) instructions for using the amount effective of active agent for increasing blood flow to ischemic cardiac tissue.

3. The kit of claim 2 further comprising a means for delivery of the active agent.

4. A method for increasing blood flow to ischemic cardiac tissue comprising the administration of an amount effective for increasing blood flow to ischemic cardiac tissue of at least one active agent comprising a sequence selected from the general formula:

R1-Arg-R2-R3-R4-His-Pro-R5,

wherein

R1 is Asp or is absent;

R2 is selected from the group consisting of Val, Ala, Ile, Pro, Lys, Norleu, and Leu;

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R3 is selected from the group consisting of Ala, Tyr, and Tyr(PO₃)₂;

R4 is selected from the group consisting of Val, Ala, Ile, Norleu, and Leu;

R5 is Phe, Ile, or is absent; and

wherein the active agent is not AII.

5. The method of claim 4 wherein the active agent is selected from the group consisting of SEQ ID NO:4, SEQ ID NO:19, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, and SEQ ID NO:42.

6. A kit for increasing blood flow to ischemic cardiac tissue, comprising:

(a) an amount effective for increasing blood flow to ischemic cardiac tissue of at least one active agent comprising a sequence selected from the general formula

R1-Arg-R2-R3-R4-His-Pro-R5,

wherein

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R1 is Asp or is absent;

R2 is selected from the group consisting of Val, Ala, Ile, Pro, Lys, Norleu, and Leu;

R3 is selected from the group consisting of Ala, Tyr, and Tyr(PO₃)₂;

R4 is selected from the group consisting of Val, Ala, Ile, Norleu, and Leu;

R5 is Phe, Ile, or is absent;

wherein the active agent is not AII; and

(b) instructions for using the amount effective of active agent for increasing blood flow to ischemic cardiac tissue.

7. The kit of claim 6 wherein the active agent is selected from the group consisting of SEQ ID NO:4, SEQ ID NO:19, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42.

8. The kit of claim 6, further comprising a means for delivery of the active agent.

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PDNC Blue #1. The essential characteristic of the dye is that it be soluble in the liquid being collected.

The individual collection apparatuses of the invention can be stored in a fluid collection kit comprising multiple tubes of the two types described above and multiple caps. The kit will normally comprise a container adapted to hold the tubes and caps in a readily accessible manner (typical of the type used in a test tube rack in which the individual tubes are inserted into holes in a rack-like device, typically made of cardboard in a commercial collection kit). The individual tubes can have built-in labels for ease of use (for example, containing spaces for patient name and date and time of collection), and written instructions adapted for the particular type of sample can be included in the box that holds the individual tubes.

The instant invention further provides kits for monitoring the effectiveness of hormone replacement therapy in a early menopausal or late menopausal woman, comprising a fluid collection device, such as those described above, and instructions for using the fluid collection device for monitoring the effectiveness of hormone replacement therapy in a menopausal woman by using the methods of the invention described above.

It is to be understood that the invention is not to be limited to the exact details of operation, or to the exact compounds, compositions, methods, procedures or embodiments shown and described, as obvious modifications and equivalents will be apparent to one skilled in the art, and the invention is therefore to be limited only by the full scope of the appended claims.

We claim:

1. A method for monitoring the effectiveness of hormone replacement therapy in a perimenopausal woman, comprising

- a. obtaining a body fluid sample from the perimenopausal woman;
- b. testing for hormonal levels in the body fluid of the perimenopausal woman at intervals of between about 7 days to about 90 days, wherein testing for hormonal

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levels comprises testing for levels of at least one of progesterone, testosterone, estradiol, follicle stimulating hormone, and estriol; and

c. adjusting levels of replacement hormone administered to the woman based on the test results.

2. A method for monitoring the effectiveness of hormone replacement therapy in a early menopausal or late menopausal woman, comprising:

- a. obtaining a body fluid sample from the perimenopausal woman;
- b. testing for hormonal levels in the body fluid of the perimenopausal woman at intervals of between about 30 days to about 180 days, wherein testing for hormonal levels comprises testing for levels of at least one of progesterone, testosterone, estradiol, follicle stimulating hormone, and estriol; and

c. adjusting levels of replacement hormone administered to the woman based on the test results.

3. The method of claim 1 wherein the body fluid is saliva.

4. The method of claim 2 wherein the body fluid is saliva.

5. A kit for monitoring the effectiveness of hormone replacement therapy in a perimenopausal woman, comprising:

- a. a fluid collection device;
- b. instructions for using the fluid collection device for monitoring the effectiveness of hormone replacement therapy in a menopausal woman according to the method of claim 1.

6. A kit for monitoring the effectiveness of hormone replacement therapy in a early menopausal or late menopausal woman, comprising:

- a. a fluid collection device;
- b. instructions for using the fluid collection device for monitoring the effectiveness of hormone replacement therapy in a menopausal woman according to the method of claim 2.

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wherein the snare cords are arranged about the pins in the loose knot pattern.

4. A knot tying device as in claim 3, wherein the pins are arranged in a pattern having a cluster of four pins at one end and a line of at least three pins extending from the cluster.

5. A knot tying device as in claim 1, wherein the capture end of each snare cord comprises a loop for receiving a suture end therethrough.

6. A knot tying device as in claim 1, wherein the pull end of each snare cord comprises a handle to permit manual grasping thereof.

7. A knot tying device as in claim 1, wherein the knot is selected from the group consisting of a square knot, a sliding surgeon's knot, a square knot, a cinch knot, a capstan knot, and a reef knot.

8. A knot tying device comprising:

a handle;

a knotting guide attached to the handle, said knotting guide including a plurality of guide pins arranged in a pre-defined pattern and a cover which can be selectively opened and closed over the pins;

a first snare cord having a capture end and a pull end arranged over the pins in a partial knot pattern; and

a second snare cord having a capture end and a pull end arranged over the pins in a knot pattern complementary to the partial knot pattern;

whereby a knot may be formed in first and second suture ends by capture of the first suture end with the capture end of the first snare cord, capture of the second suture end with the capture end of the second snare cord, pulling of the first snare cord through the pins so that the first suture end assures the partial knot pattern, pulling of the second snare cord through the pins to assure the complementary knot pattern, opening of the cover, and removal of the knot in the suture ends from the knotting guide.

9. A knot tying device as in claim 8, wherein the pins are arranged in a pattern having a cluster of four pins at one end and a line of at least three pins extending from the cluster.

10. A knot tying device as in claim 8, wherein the capture end of each snare cord comprises a loop for receiving a suture end therethrough.

11. A knot tying device as in claim 8, wherein the pull end of each snare cord comprises a handle to permit manual grasping thereof.

12. A knot tying device as in claim 8, wherein the knot is selected from the group consisting of a square knot, a sliding surgeon's knot, a square knot, a cinch knot, a capstan knot, and a reef knot.

13. A kit comprising a knot tying device as in claim 1, and a package, wherein the device is sterile and contained in the package.

14. A kit comprising:

a knot tying device including:

a first snare cord having a capture end and a pull end;

a second snare cord having a capture end and a pull end; and

instructions for use setting forth the following steps:

capturing a first suture end with the capture end of the first snare cord;

capturing a second suture end with the capture end of the second snare cord;

pulling the first snare cord through a path which defines a partial knot pattern; and

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pulling the second snare cord through a path which defines a complementary knot pattern;

whereby the suture ends are formed into a knot.

15. A method for tying a first suture end and a second suture end into a knot, said method comprising:

capturing the first suture end with a first snare cord;

capturing the second suture end with a second snare cord;

pulling the first snare cord through a path which defines a partial knot pattern; and

pulling the second snare cord through a path which defines a knot pattern complementary to the partial knot pattern;

whereby the suture ends are formed into a complete knot.

16. A method as in claim 15, wherein the first suture end and the second suture end comprise opposite ends of a single length of suture.

17. A method as in claim 16, wherein the single length of suture is anchored in tissue.

18. A method as in claim 16, wherein the single length of suture passes through penetrations on the periphery of a blood vessel puncture.

19. A method as in claim 16, wherein the single length of suture passes between blood vessels in an anastomotic attachment.

20. A method as in claim 16, wherein the single length of suture passes between a prosthetic device and tissue.

21. A method as in claim 15, wherein the capturing steps both comprise placing the suture end in a loop formed in the end of the snare cord.

22. A method as in claim 21, wherein the pulling steps both comprise drawing the snare cord through a knotting guide which holds the first snare cord and the second snare cord in a loose knot pattern prior to the pulling steps.

23. A method as in claim 22, wherein the two pulling steps are performed sequentially.

24. A method as in claim 22, wherein the two pulling steps are performed simultaneously.

25. A method as in claim 15, further comprising advancing the complete knot to engage a tissue surface.

26. A method as in claim 15, wherein the complete knot comprises a sliding knot having a rail end and a cinching end, wherein the knot is advanced by holding the rail end and pushing the knot.

27. A method as in claim 15, wherein the complete knot comprises a square knot.

28. A knotting device comprising:

a knotting guide which imparts a loose knot pattern in a first suture end and a second suture end; and

a shaft removably secured to the knotting guide, the shaft defining a distal passage for receiving one of the first and second suture ends, wherein said shaft is positioned adjacent to the loose knot after the knot has been formed and the knotting guide has been removed, so that the shaft can be advanced over the one of the first and second suture ends advance the knot along said one of the first and second suture ends.

29. A knotting device as in claim 28, wherein the knotting guide comprises:

a first snare cord having a capture end and a pull end;

a second snare cord having a capture end and a pull end; and

a knotting guide which holds the first snare cord and the second snare cord in a loose knot pattern so that a knot may be formed in a first suture end and a second suture

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in treating neurodegenerative pathologies not significantly affecting memory or learning. Preferred compounds and unit doses include those described herein above.

We claim:

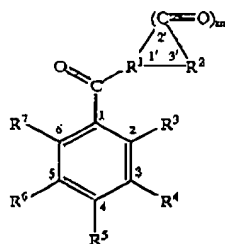
1. A method for treating schizophrenia in a subject, said method comprising administering an effective amount of a composition that comprises a first compound that enhances the stimulation of α -amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid ("AMPA") receptors in said subject and a second antipsychotic compound.

2. The method of claim 1 wherein the composition is administered orally.

3. The method of claim 1 wherein the composition is administered by injection.

4. A kit, comprising a container containing the composition of claim 1 and instructions for using the composition for treating schizophrenia in a subject.

5. A method in accordance with claim 1 wherein said first compound has the following formula, with ring vertices numbered as shown:



in which:

R^1 is a member selected from the group consisting of N and CH_3 ;

m is 0 or 1;

R^2 is a member selected from the group consisting of $(CR^8)_n$ and $C_{n-m}HR^8_{2(n-m)-3}$, in which n is 4, 5, 6, or 7, the R^8 's in any single compound being the same or different, each R^8 being a member selected from the group consisting of H and C_1-C_6 alkyl, or one R^8 being combined with either R^3 or R^7 to form a single bond linking the no. 3' ring vertex to either the no. 2 or the no. 6 ring vertices or a single divalent linking moiety linking the no. 3' ring vertex to either the no. 2 or the no. 6 ring vertices, the linking moiety being a member selected from the group consisting of CH_2 , CH_2-CH_2 , $CH=CH$, O, NH, $N(C_1-C_6 \text{ alkyl})$, $N=CH$, $N=C(C_1-C_6 \text{ alkyl})$, $C(O)$, $O-C(O)$, $C(O)-O$, $CH(OH)$, $NH-C(O)$, and $N(C_1-C_6 \text{ alkyl})-C(O)$;

R^3 , when not combined with any R^8 , is a member selected from the group consisting of H, C_1-C_6 alkyl, and C_1-C_6 alkoxy;

R^4 is either combined with R^5 or is a member selected from the group consisting of H, OH, and C_1-C_6 alkoxy;

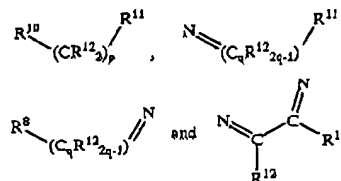
R^5 is either combined with R^4 or is a member selected from the group consisting of H, OH, C_1-C_6 alkoxy, amino, mono(C_1-C_6 alkyl)amino, di(C_1-C_6 alkyl)amino, and CH_2OR^9 , in which R^9 is a member selected from the group consisting of H, C_1-C_6 alkyl, an aromatic carbocyclic moiety, an aromatic heterocyclic moiety, an aromatic carbocyclic alkyl moiety, an aromatic heterocyclic alkyl moiety, and any such moiety substituted with one or more members selected from the group consisting of C_1-C_3 alkyl, C_1-C_3 alkoxy,

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hydroxy, halo, amino, alkylamino, dialkylamino, and methylenedioxy;

R^6 is either H or CH_2OR^9 ;

R^4 and R^5 when combined form a member selected from the group consisting of



in which

R^{10} is a member selected from the group consisting of O, NH and $N(C_1-C_6 \text{ alkyl})$;

R^{11} is a member selected from the group consisting of O, NH and $N(C_1-C_6 \text{ alkyl})$;

R^{12} is a member selected from the group consisting of H and C_1-C_6 alkyl, and when two or more R^{12} 's are present in a single compound, such R^{12} 's are the same or different;

p is 1, 2, or 3; and

q is 1 or 2; and

R^7 , when not combined with any R^8 , is a member selected from the group consisting of H, C_1-C_6 alkyl, and C_1-C_6 alkoxy.

6. A method in accordance with claim 5 in which R^2 is a member selected from the group consisting of $(CHR^8)_n$ and $C_{n-m}HR^8_{2(n-m)-3}$, and R^3 is a member selected from the group consisting of H, C_1-C_6 alkyl, and C_1-C_6 alkoxy.

7. A method in accordance with claim 5 in which R^2 is a member selected from the group consisting of $(CHR^8)_n$ and $C_{n-m}HR^8_{2(n-m)-3}$, and one R^8 is combined with either R^3 or R^7 to form a single bond linking the no. 3' ring vertex to either the no. 2 or the no. 6 ring vertices or a single divalent linking moiety linking the no. 3' ring vertex to either the no. 2 or the no. 6 ring vertices, the linking moiety being a member selected from the group consisting of CH_2 , CH_2-CH_2 , $CH=CH$, O, NH, $N(C_1-C_6 \text{ alkyl})$, $N=CH$, $N=C(C_1-C_6 \text{ alkyl})$, $C(O)$, $O-C(O)$, $C(O)-O$, $CH(OH)$, $NH-C(O)$, and $N(C_1-C_6 \text{ alkyl})-C(O)$.

8. A method in accordance with claim 5 in which R^2 is a member selected from the group consisting of $(CHR^8)_n$ and $C_{n-m}HR^8_{2(n-m)-3}$, and one R^8 is combined with either R^3 or R^7 to form a single bond linking the no. 3' ring vertex to either the no. 2 or the no. 6 ring vertices or a single divalent linking moiety linking the no. 3' ring vertex to either the no. 2 or the no. 6 ring vertices, the linking moiety being a member selected from the group consisting of CH_2 , CH_2-CH_2 , $CH=CH$, O, NH, $C(O)$, and $CH(OH)$.

9. A method in accordance with claim 5 in which R^2 is a member selected from the group consisting of $(CHR^8)_n$ and $C_{n-m}HR^8_{2(n-m)-3}$, and one R^8 is combined with either R^3 or R^7 to form a single divalent linking moiety linking the no. 3' ring vertex to either the no. 2 or the no. 6 ring vertices, the linking moiety being a member selected from the group consisting of CH_2 , O, NH, $C(O)$, and $CH(OH)$.

10. A method in accordance with claim 5 in which m is zero, R^2 is a member selected from the group consisting of $CHR^8-CH_2-CH_2-CH_2$ and $CHR^8-CH_2-CH_2-CH_2$, in which R^8 is combined with R^7 to form a single divalent linking moiety linking the 2 and 3' ring vertices, the linking moiety being a member selected from the group consisting of CH_2 , O, NH, $C(O)$, and $CH(OH)$.

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regulatory pathway or a gene product, is an expensive multistep process, with biochemical or molecular cloning approaches. Applying the immunohistochemical procedure as described, equipped with a broad spectrum antibody to a kinetic regulatory protein, a straight forward identification of defects in its translocation are identified first. Next, a comparison of structural components of a normal translocator versus defective non-translocator will provide a clue regarding those transcription factors which are essential to induce intracellular translocation of a nuclear binding component.

What I claim is:

1. An immunohistochemical method to detect normal and abnormal intracellular movement of a kinetic cellular component from one cellular compartment to another, comprising the steps of:

- a) pre-coating the glass slides with a solution containing a ligand specific to the kinetic component to be examined, a control ligand or no ligand; wherein a specific ligand is an agent or a compound which upon incubation with the said kinetic component effects its intracellular movement from one compartment to another; wherein a control ligand does not upon incubation effect an intracellular movement of the said kinetic component;
- b) imprinting tumor tissue, thaw mounting cryosections of normal or tumor tissue or smearing core-needle biopsies or cell suspensions on uncoated or said pre-coated glass slides;
- c) incubating the slides in moist chamber to allow for ligand binding;
- d) fixing the incubated tissue;
- e) detecting the kinetic component by binding an antibody to the said kinetic component;
- f) determining the normal or abnormal movement of the said kinetic component by comparing its cellular location when incubated with or without the specific ligand.

2. The immunohistochemical method of claim 1 whereby breast tumors are classified based upon the presence in tumor cells of normal or defective estrogen receptors,

wherein, estrogen receptor is the kinetic component;

wherein, normal estrogen receptor upon incubation with estrogen receptor-specific ligand moves from the cytoplasm to the nuclear compartment of the cell and the tumor with said normal receptors is classified as Tr+L;

wherein, defective estrogen receptor upon incubation with estrogen receptor-specific ligand fails to move from cytoplasm to the nuclear compartment of the cell and the tumor with said defective estrogen receptors are classified as Tr(-);

wherein, defective estrogen receptors upon incubation with no ligand or with control ligand moves from the cytoplasm to the nuclear compartment of the cell and the tumor with said defective estrogen receptors is classified as Tr+NL;

wherein, the detection of normal and defective estrogen receptor is accomplished by:

- a) imprinting tumor tissue, thaw mounting cryosections of tumor or normal tissue, smearing core-needle biopsy or cell suspension on estrogen receptor-specific ligand coated or control ligand coated slides;
- b) incubating the slides in moist chamber to allow ligand binding;

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- c) fixing the incubated tissue;
- d) detecting the estrogen receptor by binding an antibody to estrogen receptor;
- e) determining the percentage of cells with normal or defective estrogen receptors and classifying as normal or Tr+L, defective or Tr(-), or as defective or Tr+NL variety.

3. The immunohistochemical method of claim 2 whereby breast tumor patients are selected for hormonal or other non-hormonal modes of treatments;

wherein patients with tumors of Tr+L variety containing normal estrogen receptors are predicted to be responders to hormonal therapy and thereby selected for hormonal treatments;

wherein patients with tumors of Tr(-) and/or Tr+NL varieties containing defective estrogen receptors are predicted to be non-responsive to hormonal modes of therapy and thereby selected for non-hormonal modes of therapies.

4. An immunohistochemical method of screening for the ligand specific for a kinetic cellular component

wherein a specific ligand is an agent or a compound that upon incubation with the kinetic cellular component effects its intracellular movement from one compartment to another;

wherein estrogen receptor is a kinetic cellular component and specific ligand upon incubation effects the movement of estrogen receptor from the cytoplasm to the nuclear compartment of the cell ligand screening comprise the steps of:

- a) precoating glass slides with a solution of ligand known to be specific to estrogen receptor, the solution to be screened for the presence of a specific ligand or a solution of control ligand;
- b) imprinting tumor tissue with normal estrogen receptor, thaw-mounting cryosections of tumor or normal tissue with estrogen receptor, smearing cell suspension from estrogen receptor containing tissue onto said pre-coated slides;
- c) incubating the slides in a moist chamber to allow ligand binding;
- d) fixing the incubated tissue;
- e) detecting the estrogen receptor by binding an antibody specific to estrogen receptor;
- f) determining the cellular location of estrogen receptor in the presence vs absence of the solution being screened for ligand.

5. The immunohistochemical ligand screening method of claim 4, whereby screening for an inhibitor-ligand is accomplished,

wherein an inhibitor-ligand is an agent or a compound which when combined with an estrogen receptor specific ligand, prevents the estrogen receptor from moving from the cytoplasm to the nuclear compartment of the cell;

and the method of screening further comprising the steps of:

- a) precoating glass slides with a solution containing estrogen receptor specific ligand, a solution of control ligand or a solution of specific ligand mixed with the solution to be screened for an inhibitor-ligand;
- b) following steps b-e specified for claim 4;
- c) determining the location of estrogen receptor in the presence of specific ligand and in the mixture containing both the specific ligand and the ligand being examined.

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6. An immunohistochemical method for screening for monoclonal anti-estrogen receptor antibodies with exclusive specificity for either normal Tr+L, defective Tr(-) or defective Tr+NL estrogen receptors, wherein to normal/Tr+L receptors are those which need binding to the specific ligand to move from cytoplasm to the nuclear compartment of the cell;

wherein defective Tr(-) estrogen receptors are those which do not move from cytoplasm to the nuclear compartment even upon binding to estrogen receptor specific ligand;

wherein defective Tr+NL estrogen receptors are those which will move from cytoplasm to nuclear compartment of the cell even in the absence of estrogen receptor specific ligand;

wherein the antibody screening comprises the steps of:

- a) imprinting on glass slide, or thaw mounting cryosections of normal tissue or tumor tissue with Tr+L estrogen receptors, Tr(-) estrogen

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b) air drying the tissue followed by fixing the tissue on the slide;

c) contacting the fixed tissue with solution containing monoclonal antiestrogen receptor antibody;

d) determining the antibody binding to Tr+L, Tr(-) or Tr+NL estrogen receptors or to all three by immunohistochemical staining techniques.

7. A kit intended for the purpose as described in claim 1 and comprising any of the following either singly or in combination

a) Ligand-coated glass slides

b) saline or buffer-coated glass slides

c) tissue or tumor imprinted glass slides

d) glass slides smeared with cell suspensions

e) instructions with procedural details described in claim 1.

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TABLE 5-continued

| Treatment group | Spleen weight (\bar{x} = animal died untimely) | |
|----------------------------|--|--|
| | Spleen weight (g) after 4 weeks treatment | Spleen weight (g) after 2 weeks with no treatment |
| Median value CY + G-CSF | 0.492 | 0.261 |
| | 0.483 | 0.153 |
| | 0.486 | 0.273 |
| | 0.491 | 0.224 |
| | 0.263 | 0.177 |
| | 0.218 | 0.228 |
| | 0.225 | 0.208 |
| | 0.324 | 0.218 |
| Median value | 0.254 | 0.232 |
| | 0.257 | 0.213 |

TABLE 6

| Treatment group | Animal number | WBC ($\times 10^6/\mu$) Time (weeks) | | | | |
|----------------------------|------------------|---|------|------|-------|-------|
| | | 0 | 1 | 2 | 3 | 4 |
| Control | 1 | 5.6 | 5.2 | 9.4 | 3.3 | 6.6 |
| | 2 | 10 | 6.3 | 6.9 | † | † |
| | 3 | 7 | 4.8 | 7 | 3.4 | 6.8 |
| | 4 | 7.6 | 7.6 | 7.7 | 3.9 | 7.7 |
| | 5 | 9.1 | 6 | 11 | 3.5 | 6.3 |
| | 6 | 8.4 | 5.1 | 9.5 | 6.2 | 7.5 |
| | 7 | 2.5 | 2.3 | 5.3 | 5 | 5.7 |
| | 8 | 9.5 | 7.3 | 12.2 | 8.5 | 13.7 |
| | 9 | 6.3 | 4.3 | 8.8 | 4.2 | 10.1 |
| | 10 | 6 | 5.9 | 8.5 | 5.9 | 8 |
| Median value CY alone | 1 | 7.20 | 5.48 | 8.63 | 4.88 | 8.04 |
| | 2 | 4.8 | 2.9 | 5 | 7.6 | 4 |
| | 3 | 9 | 7.1 | 5.3 | 2.7 | 2.8 |
| | 4 | 8.6 | 5.9 | 7.3 | 5.2 | 7 |
| | 5 | 6.3 | 4.8 | 6.3 | 6.1 | 4.8 |
| | 6 | 5.3 | 4.5 | 4.9 | 8.6 | 2.9 |
| | 7 | 6.4 | 5 | 5.1 | 6.7 | 4.1 |
| | 8 | 6.8 | 4.7 | 4.9 | 5 | 3.6 |
| | 9 | 5.6 | 5.7 | 4.8 | 4.1 | 3.7 |
| | 10 | 5.8 | 4.7 | 5.9 | 4.1 | 7.1 |
| Median value G-CSF + CY | 1 | 9.4 | 6 | 7.1 | 4 | 2.8 |
| | 2 | 6.80 | 5.13 | 5.66 | 5.41 | 4.28 |
| | 3 | 6.1 | 11.7 | 27.7 | 33.4 | 39.4 |
| | 4 | 6.8 | 12.4 | 46.5 | 43.7 | 30.5 |
| | 5 | 5.6 | 11.2 | 30.3 | 58.78 | 40.6 |
| | 6 | 4.3 | 11.7 | 35.3 | 51.4 | 40.7 |
| | 7 | 6.2 | 22.4 | 62.2 | 55.4 | 66.7 |
| | 8 | 6.2 | 12.6 | 49.2 | 84.2 | 85.4 |
| | 9 | 7.1 | 16.8 | 84.8 | 105.4 | 102.3 |
| | 10 | 8 | 18.6 | 82 | 117.6 | 70.1 |
| | 11 | 3 | 5.4 | 42.7 | 15.8 | 33.3 |
| | 12 | 4.6 | 18.2 | 62.8 | 69 | 33.2 |
| | 13 | 5.8 | 10.9 | 29.1 | 40 | 33.5 |
| | 14 | 5 | 12.7 | 43.8 | 51.9 | 28.1 |
| | 15 | 5.8 | 14.6 | 28.8 | 33.4 | 20 |
| | 16 | 6.2 | 10.2 | 50 | 36.8 | 45.4 |

TABLE 6-continued

| Treatment group | Animal number | Leukocyte number (WBC) (\bar{x} = animal died untimely) | | | | |
|-----------------|------------------|---|-------|-------|-------|-------|
| | | WBC ($\times 10^6/\mu$) Time (weeks) | | | | |
| 15 | 15 | 8.6 | 20.2 | 30 | 36.2 | 25.3 |
| | 16 | 5.9 | 12.8 | 26.8 | 45.5 | 54.8 |
| 10 | Median value | 6.00 | 14.04 | 48.08 | 55.16 | 44.60 |
| | CY + G-CSF | 1 | 6.7 | 6.1 | 6.9 | 9.5 |
| 15 | 2 | 9.1 | 7.1 | 11.08 | 11.6 | 3.78 |
| | 3 | 9.1 | 11.1 | 4.5 | 9.6 | 5.9 |
| 20 | 4 | 6.9 | 5.2 | 10.4 | 16.6 | 5 |
| | 5 | 6.3 | 3.9 | 7 | # | 11.6 |
| 25 | 6 | 9.6 | 8.3 | 5 | 6.9 | 4.1 |
| | 7 | 6.6 | 6.3 | 8.6 | 7.1 | 7.5 |
| 30 | 8 | 6.1 | 4.4 | 5.4 | 3.8 | 3.18 |
| | 9 | 10.3 | 7.6 | 4.8 | 4.2 | 6.2 |
| 35 | 10 | 8.2 | 6.5 | 5.4 | 6.2 | 4.3 |
| | Median value | 7.89 | 6.65 | 6.91 | 8.39 | 6.42 |

What is claimed is:

1. A method of treating a disease requiring peripheral stem cell transplantation in a patient in need of such treatment, comprising administering to the patient a hematopoietic stem cell mobilizing-effective amount of G-CSF; and thereafter administering to the patient a disease treating-effective amount of at least one chemotherapeutic agent.

2. The method of claim 1, wherein the disease is a tumor disease.

3. The method of claim 1, wherein the G-CSF is recombinant G-CSF.

4. The method of claim 1, wherein the at least one chemotherapeutic agent opens the endothelial barrier of the patient to render the endothelial barrier permeable for stem cells.

5. The method of claim 1, wherein the at least one chemotherapeutic agent is cyclophosphamide.

6. The method of claim 1, wherein the G-CSF is administered once per day over 2-3 consecutive days, and the chemotherapeutic agent is administered immediately after the final administration of G-CSF, or on a fourth consecutive day.

7. A pharmaceutical kit, comprising

a first component comprising G-CSF;

a second component comprising at least one chemotherapeutic agent; and

a third component comprising instructions for the administration of the G-CSF prior to the onset of administration of the at least one chemotherapeutic agent.

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has been snapped into the locked position, thereby securing the curling ribbon 1200 to the strand of hair 70.

As shown in FIG. 14, a lower portion of the right hand side 1212 of the curling ribbon 1200 is first folded over the strand 70, and then the left hand side 1214 is folded over the right hand side 1212 in the direction of arrow 1215 so as to envelop the strand 70 inside the curling ribbon 1200. The curling ribbon 1200 is then wound around a curling mandrel, and the mandrel is withdrawn in the same way as illustrated in FIGS. 7 through 9 with respect to curling ribbon 400. When the ribbon 1200 is unwrapped from the curled hair, you use your fingers to unsnap the locking comb 1280 to release the hair strand 70, which unsecures the curling ribbon 1200 from the hair.

As shown in FIG. 12, the locking comb 1280 may be attached to the curling ribbon 1200 by base stiffener 1240 of wire extending through holes 1284. Alternatively, the locking comb 1280 may be attached to the curling ribbon 1200 by sewing it through holes 1284 to the fabric 1210, and in that event the base stiffener 1240 may optionally be omitted. In either event the end stiffener 1250 may optionally be omitted from the curling ribbon when a locking comb 1280 is used.

Modifications to the embodiments described above may be made without departing from the basic spirit of the present invention. Accordingly, it will be appreciated by those skilled in the art that, within the scope of the appended claims, the invention may be practiced other than has been specifically described herein.

I claim:

1. A method for curling hair comprising the following steps:

Isolating a strand of hair that the subject desires to be curled;

Providing a curling ribbon comprising a ribbon of fabric having a plurality of bendable stiffening elements extending longitudinally thereof;

Placing the curling ribbon adjacent the strand of hair and wrapping it around the strand to envelop substantially the entire hair strand;

Securing the curling ribbon to hair at or near the base of the strand;

Providing a curling mandrel of generally cylindrical shape having a diameter the size of the desired curls, and placing it adjacent the base of the hair strand to which the curling ribbon has been secured;

Wrapping the curling ribbon helically around the curling mandrel until substantially the entire strand of hair is coiled around the curling mandrel within the curling ribbon;

Withdrawing the curling mandrel axially from within the coil without uncoiling; the strand;

Applying a curling agent to the strand; and

Permitting the strand to remain coiled within the curling ribbon until the curling agent has curled the strand of hair.

2. The method of claim 1, wherein the curling agent is a setting composition.

3. The method of claim 2, wherein the setting composition is applied to the strand before the curling ribbon is wrapped around the strand.

4. The method of claim 1, wherein the curling agent is a permanent waving composition.

5. The method of claim 4, wherein the setting composition is applied to the strand after the curling mandrel has been withdrawn.

6. The method of claim 1, wherein the curling ribbon is secured to the strand of hair by a spring-loaded clip.

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7. The method of claim 1, wherein the curling ribbon further comprises a comb at its base end and the curling ribbon is secured to the strand of hair by said comb.

8. The method of claim 7, wherein said comb is a locking comb of flexible metal.

9. The method of claim 1, further comprising the step of: Removing the curling ribbon from around the strand and unsecuring it from the strand, so that the curled hair strand may be combed out.

10. The method of claim 1, further comprising the steps of:

Isolating additional strands of hair on the subject's head; Providing an additional curling ribbon for applying to each of said additional strands of hair;

Placing one the additional curling ribbons adjacent each of the additional strands of hair and wrapping it around the strand to envelop substantially the entire hair strand;

Securing the additional curling ribbon to hair at or near the base of the strand;

Placing said curling mandrel adjacent the base of each of the additional hair strands to which one of the additional curling ribbons has been secured;

Wrapping the curling ribbon helically around the curling mandrel until substantially the entire strand of hair is coiled around the curling mandrel within the curling ribbon;

Withdrawing the curling mandrel axially from within the coil without uncoiling; the strand;

Applying a curling agent to the strand; and

Permitting each of the additional strands of hair to remain coiled within each of the additional curling ribbons until the curling agent has curled the strands of hair.

11. A curling ribbon adapted for use in the method of claim 1, comprising

A ribbon of fabric having a base end and a distal end;

A pair of edge stiffeners extending longitudinally along each edge; and

A longitudinal stiffener extending parallel to said edge stiffeners between them.

12. The curling ribbon of claim 11, further comprising a comb secured to the base end of said ribbon of fabric.

13. The curling ribbon of claim 11, further comprising a base stiffener.

14. The curling ribbon of claim 11, further comprising a comb secured to the base stiffener.

15. The curling ribbon of claim 11, further comprising an end stiffener.

16. The curling ribbon of claim 11, wherein said ribbon of fabric is between about 5 and 7 centimeters wide.

17. The curling ribbon of claim 11, wherein said ribbon of fabric is between about 40 and 75 centimeters long.

18. The curling ribbon of claim 11, wherein said ribbon of fabric is brightly colored.

19. The curling ribbon of claim 11, wherein said ribbon of fabric bears a decorative design.

20. A kit for use in performing the method of claim 1, comprising at least 20 curling ribbons, a first curling mandrel, and instructions.

21. The kit of claim 20, further comprising at least one additional curling mandrel having a different diameter from said first curling mandrel.

22. The kit of claim 20, further comprising a curling agent.

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TABLE 1-continued

| Monkey | Fe D 0 µg/ml | Fe D 7 µg/ml | TransFe sat % Day 0 | TransFe sat % Day 7 | Toxicity Liver & Kidney |
|--------|-----------------|-----------------|---------------------------|---------------------------|-------------------------------|
| N11 | 135 | 1 | 33 | -2 | L+/K- |
| 738PR | | 1 | | -4 | L+/K+ |

Mean 109 ± 18 2 ± 2 34 ± 4 -1.2 ± 2

p < 0.001 p < 0.0001

Pathology

the liver- (L+) swelling of hepatocytes, (L++) degeneration, (L+++) lipidosis (#73, #105), regeneration (885M)
the kidneys- (K+) multifocal casts, (K++) glomerulonephritis (#73, #105), hemosiderin deposits (K-) no toxicity
No brain changes (substantia nigra, cerebellum- Purkinje cells)

The results of this study shows that a specific chelator of ferrous iron can prevent vasospasm in a primate model of SAH. In this primate model of SAH-related vasospasm, the reliability of producing spasm in untreated animals is 98% [Pluta et al., J. Neurosurg. 77:740-748 (1992)]. Six animals receiving 70 mg/kg/day of dipyrindyl had no arteriographic vasospasm. The serum iron was markedly reduced in the drug-treated animals on day 7, as compared to day 0, confirming chelation of the plasma iron in the treated animals. The percent saturation for the endogenous chelator transferrin was decreased in treated animals, suggesting that dipyrindyl strongly chelates iron. Furthermore, toxicity at the effective dose is mild.

What is claimed:

1. A method of preventing or treating cerebral vasospasm comprising:

internally administering to a human who has suffered subarachnoid hemorrhage an effective amount of an iron chelator which preferentially chelates ferrous iron over ferric iron and which competes with nitric oxide binding to ferrous iron, thereby interfering with the nitric oxide sink effect,

wherein said iron chelator is selected from the group consisting of 2,2'-dipyridyl and 1,10-phenanthroline.

2. The method of claim 1, wherein said iron chelator is internally administered through a route selected from the group consisting of a continuous IV, intravenous bolus, and oral.

3. The method of claim 1, wherein said iron chelator is internally administered to the human within 7 days of the subarachnoid hemorrhage.

4. The method of preventing or treating vasospasm of claim 1, wherein the said iron chelator further penetrates the blood-brain barrier and acts intracellularly.

5. The method of claim 2, wherein said route is intravenous bolus.

6. The method for the prevention or treatment of vasospasm of claim 1, wherein said iron chelator is 2,2' dipyrindyl.

7. The method of claim 6, wherein said iron chelator is internally administered at a dose of 20-100 mg/kg/day.

8. A kit or labeled container for the prevention or treatment of cerebral vasospasm in a human who has suffered subarachnoid hemorrhage, comprising:

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a container;

an effective amount of an iron chelator in said container which preferentially chelates ferrous iron over ferric iron and competes with nitric oxide binding to said ferrous iron thereby interfering with the nitric oxide sink effect; and

instructions either secured to said container or associated with said kit which give directions on how to use said iron chelator for prevention or treatment of cerebral vasospasm in said human who has suffered subarachnoid hemorrhage, wherein said directions provide for internally administering said effective amount of said iron chelator to said human,

wherein said iron chelator is selected from the group consisting of 2,2'-dipyridyl and 1,10-phenanthroline.

9. The kit or labeled container for the prevention or treatment of vasospasm of claim 8, wherein said iron chelator further penetrates the blood-brain barrier and acts intracellularly.

10. The kit or labeled container for the prevention or treatment of vasospasm of claim 8, wherein said iron chelator is 2,2' dipyrindyl.

11. A pharmaceutical composition for the prevention or treatment of cerebral vasospasm comprising:

an effective amount of an iron chelator which preferentially chelates ferrous iron over ferric iron and competes with nitric oxide binding to said ferrous iron thereby interfering with the nitric oxide sink effect and wherein said chelator is effective for preventing or treating cerebral vasospasm when administered internally to said human who has suffered subarachnoid hemorrhage; and

a pharmaceutically acceptable carrier,

wherein said iron chelator is selected from the group consisting of 2,2'-dipyridyl and 1,10-phenanthroline.

12. The pharmaceutical composition for the prevention or treatment of vasospasm of claim 11, wherein said iron chelator is 2,2' dipyrindyl.

13. The pharmaceutical composition for the prevention or treatment of vasospasm of claim 11, wherein said iron chelator further penetrates the blood-brain barrier and acts intracellularly.

14. The pharmaceutical composition of claim 12, wherein said ferrous iron chelator is present in a concentration to allow the administration of an amount of 20-100 mg/kg/day.

15. The pharmaceutical composition of claim 12, wherein said ferrous iron chelator is present in concentration 0.5-50 mg/ml.

16. The pharmaceutical composition of claim 12, wherein said ferrous iron chelator is present in concentration 5-25 mg/ml.

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EXAMPLE 5

In this experiment, milrinone was evaluated for its capability to induce erection in the anesthetized male cat. Adult male cats (3.5 to 4.7 kg) were initially sedated with ketamine and then anesthetized and maintained with supplemental doses of pentobarbital administered through a polyethylene catheter inserted into the left external jugular vein. After exposure of the pubic area, a 25-gauge needle was placed into the left corpus cavernosum for measurement of intracavernous pressure. Milrinone (Sigma Chemical, St. Louis, Mo.) was dissolved in 20% dimethylsulfoxide (DMSO) and diluted with 0.9% NaCl. All drug solutions were stored in a freezer in amber bottles; working solutions were prepared on a frequent basis and kept on crushed ice. Vehicle and various doses of milrinone (3, 10, 30 and 100 μ g) were administered by direct injection into the right corpus cavernosum using a 30-gauge needle. Doses of zaprinast (3, 10, 30 and 100 μ g) were also given for purposes of comparison (see Table). All injections were made using a total volume of 200 μ l. At the end of the experiment, each animal received a control drug solution containing 1.65 mg papaverine, 25 μ g phentolamine, and 0.5 μ g of prostaglandin E_1 to establish maximal response in each subject.

Intracavernosal injections of milrinone and zaprinast caused dose dependent increases in cavernosal pressure and penile length. The maximal increase in cavernosal pressure in response to milrinone was an approximate five-fold increase from a baseline value of 20 ± 2 mmHg (zaprinast was similar). The 100 μ g dose of milrinone produced $81 \pm 7\%$ of the erectile response elicited by the standard control combination. The erectile response resulting from the 100 μ g dose of milrinone lasted 20 ± 6 minutes, longer than the response to a 100 μ g dose of zaprinast (duration 14 ± 1 minutes). These results are illustrated in FIGS. 2 and 3, bar graphs showing measured intracavernosal pressure (FIG. 2) and penile length (FIG. 3) following intracavernosal injections of milrinone (3 to 30 μ g). (Data in the figures and in the Table are shown as mean \pm standard error of the mean for n experiments per dose; "Triple" denotes the response to the control drug solution.)

The duration of the erectile response increased in a dose-dependent manner with increasing doses of milrinone, up to a maximum of 20 ± 6 minutes at 100 μ g, the highest dose tested. Intracavernosal injection of milrinone did not produce significant decreases in systemic arterial pressure, except for a small effect at the highest (100 μ g) dose, causing a decrease of 8 ± 3 mm Hg. This was much smaller than the 34 ± 8 mm Hg decrease in systemic arterial pressure caused by the standard control combination. These results are presented in Table 1.

TABLE 1

| Duration of Erectile Response and Change in Systemic Arterial Pressure (Δ SAP) | | | |
|---|-----------------|--------------------|----------------------|
| Drug | Dose (μ g) | Duration (Minutes) | Δ SAP (mm Hg) |
| Milrinone | 3 | 7 ± 2 | 0 ± 0 |
| Milrinone | 10 | 9 ± 1 | 0 ± 0 |
| Milrinone | 30 | 14 ± 3 | -4 ± 3 |
| Milrinone | 100 | 20 ± 6 | -8 ± 3 |
| Triple | | $38 \pm 10^{**}$ | -34 ± 8 |
| Zaprinast | 3 | 7 ± 5 | -1 ± 1 |
| Zaprinast | 10 | 10 ± 3 | -1 ± 1 |
| Zaprinast | 30 | 12 ± 3 | -1 ± 3 |

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TABLE 1-continued

| Duration of Erectile Response and Change in Systemic Arterial Pressure (Δ SAP) | | | |
|---|-----------------|--------------------|----------------------|
| Drug | Dose (μ g) | Duration (Minutes) | Δ SAP (mm Hg) |
| Zaprinast | 100 | 14 ± 1 | -2 ± 2 |
| Triple | | $33 \pm 7^{**}$ | $-54 \pm 6^{**}$ |

FIG. 4 compares the dose response curves obtained from experiments in cats treated with milrinone with the dose response curves obtained from experiments in cats treated with zaprinast (data shown as mean \pm standard error of the mean; "n" is the number of experiments for each dose). When compared on a molar basis, the dose-response curves for milrinone and zaprinast were similar, indicating that the two PDE inhibitors had similar activity when administered intracavernosally. These results suggest that a selective Type III phosphodiesterase inhibitor, when administered locally, can induce significant increases in erectile response in a mammalian male. The same or greater effects are expected upon administration of a urethral suppository.

What is claimed is:

1. A method for treating erectile dysfunction in a male individual, comprising administering to the individual a therapeutically effective amount of a pharmaceutical formulation comprised of a Type III phosphodiesterase inhibitor or a pharmaceutically acceptable salt, ester, amide or prodrug thereof, wherein the formulation is administered transurethurally, topically, or transdermally.

2. The method of claim 1, wherein the formulation further comprises a pharmaceutically acceptable carrier.

3. The method of claim 1, wherein the Type III phosphodiesterase inhibitor is selected from the group consisting of bipyridines, imidazolones, imidazolines, dihydropyridazinones, dihydroquinolones, mixed Type III-Type IV inhibitors, anagrelide, bemoradan, ibudilast, isomazole, lixazinone, motapizone, olprinone, phthalazinol, pimobendan, quazinone, siguazodan and trequinsin.

4. The method of claim 3, wherein the Type III phosphodiesterase inhibitor is a bipyridine.

5. The method of claim 4, wherein the bipyridine is selected from the group consisting of amrinone and milrinone.

6. The method of claim 3, wherein the Type III phosphodiesterase inhibitor is an imidazolone.

7. The method of claim 6, wherein the imidazolone is selected from the group consisting of piroximone and enoximone.

8. The method of claim 3, wherein the Type III phosphodiesterase inhibitor is an imidazoline.

9. The method of claim 8, wherein the imidazoline is selected from the group consisting of imazodan and 5-methyl imazodan.

10. The method of claim 3, wherein the Type III phosphodiesterase inhibitor is a dihydropyridazinone.

11. The method of claim 10, wherein the dihydropyridazinone is selected from the group consisting of indolidan and LY 181512.

12. The method of claim 3, wherein the Type III phosphodiesterase inhibitor is a dihydroquinolinone.

13. The method of claim 12, wherein the dihydroquinolinone is selected from the group consisting of cilostamide, cilostazol, vesnarinone and OPC 3911.

14. The method of claim 3, wherein the Type III phosphodiesterase inhibitor is a mixed Type III-Type IV phosphodiesterase inhibitor.

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58. The formulation of claim 52, wherein the additional active agent is selected from the group consisting of nitrovasodilators, alpha receptor blocking agents, ergot alkaloids, naturally occurring, semisynthetic and synthetic prostaglandins; vasoactive intestinal peptide; and combinations thereof.

59. The formulation of claim 58, wherein the additional active agent is a prostaglandin.

60. The formulation of claim 59, wherein the additional active agent is PGE₁.

61. The formulation of claim 58, wherein the additional active agent is an alpha receptor blocking agent.

62. The formulation of claim 61, wherein the additional active agent is prazosin.

63. A Pharmaceutical formulation for treating erectile dysfunction, comprising a therapeutically effective amount of a Type III phosphodiesterase inhibitor or a pharmaceutically acceptable salt, ester, amide or prodrug thereof, and a pharmaceutically acceptable carrier suitable for administration of a drug to the skin or mucosal tissue.

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64. The formulation of claim 63, wherein the formulation comprises an ointment, cream, gel or lotion.

65. A kit for treating erectile dysfunction, comprising: a pharmaceutical formulation comprising a Type III phosphodiesterase inhibitor or a pharmaceutically acceptable salt, ester, amide or prodrug thereof; a means for locally administering the formulation; a container for housing the formulation and drug delivery means; and instructions for using the drug delivery means to administer the formulation within the context of a dosing regimen effective to treat erectile dysfunction.

66. The kit of claim 65, wherein the means for administering the agent is a transurethral drug delivery device.

67. The kit of claim 65, further including a flexible, adjustable venous flow control (VFC) device and instructions for using the VFC device.

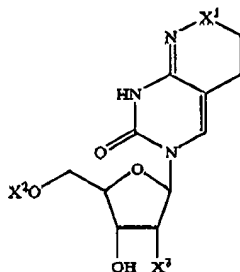
* * * * *

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We claim:

1. A compound having the structure:



where X^1 is selected from the group consisting of O, S, N-alkyl, N⁺-dialkyl and N-benzyl; X^2 is selected from the group consisting of triphosphate (P_3O_9)⁴⁻, diphosphate (P_2O_6)³⁻ and thiotriphosphate (P_3O_8S)⁴⁻; and X^3 is selected from the group consisting of H, NH₂, F and OR, where R is H, methyl, allyl or alkaryl.

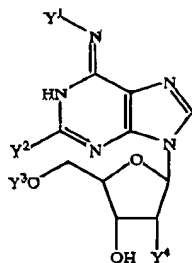
2. A compound according to claim 1, wherein X^1 is O.3. A compound according to claim 2, wherein, X^2 is triphosphate, and X^3 is H or OH.

4. A method of mutating a nucleic acid sequence, comprising replicating a template sequence in the presence of a nucleoside triphosphate analogue in accordance with claim 1, so as to form non-identical copies of the template sequence having one or more nucleoside phosphate analogue residues.

5. A method according to claim 4, comprising replicating a template sequence in the presence of deoxyP triphosphate, so as to form non-identical copies of the template sequence having one or more dP nucleotide residues.

6. A method according to claim 4, further comprising wherein the template sequence is replicated in the presence of one or more additional nucleoside triphosphates.

7. A method according to claim 4, further comprising wherein the template sequence is replicated in the presence of a second compound having the structure:



where Y^1 is selected from the group consisting of OH, O-alkyl, NH₂ and N(Alkyl)₂; Y^2 is selected from the group

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consisting of H and NH₂; Y^3 is selected from the group consisting of triphosphate (P_3O_9)⁴⁻, diphosphate (P_2O_6)³⁻ and thiotriphosphate (P_3O_8S)⁴⁻; and Y^4 is selected from the group consisting of H, NH₂, F and OR, where R is H, methyl, allyl or alkaryl.

8. A method according to claim 4, further comprising wherein the template sequence is replicated in the presence of at least one member of the group consisting of 2'-deoxy-8-hydroxyguanosine 5'-triphosphate, 2-amino-9-(2-deoxy-β-D-erythropentofuranosyl)-6-methoxyaminopurine 5'-triphosphate, and O²-ethylthymidine triphosphate.

9. A method according to claim 4, further comprising the step of replicating the non-identical copies of the template sequence in the presence of the four normal dNTPs, but in the absence of analogues thereof, to form further non-identical copies of the template sequence comprising only the four normal deoxynucleotides.

10. A method according to claim 4, wherein the replication of the template sequence, and/or the replication of the non-identical copies thereof, is achieved by means of PCR.

11. A method according to claim 4, further comprising wherein the template sequence is replicated in the additional presence of the four normal deoxynucleotides.

12. A method according to claim 5, wherein the template sequence is replicated in the presence of 1 μM to 600 μM 6-(2-deoxy-β-D-erythropentofuranosyl)-3,4 dihydro-8H-pyrimido [4,5-c][1,2]oxazine-7-one 5' triphosphate.

13. A method according to claim 5, wherein the template sequence is replicated in the presence of 1 μM to 600 μM 2'-deoxy-8-hydroxyguanosine 5'-triphosphate.

14. A kit for performing the method of claim 4, comprising said nucleoside triphosphate analogue means for replicating a template sequence so as to incorporate the nucleoside monophosphate portion of the nucleoside triphosphate analogue into non-identical copies of the template sequence, and instructions for use according to said method.

15. A kit according to claim 14, wherein the nucleoside triphosphate analogue is 6-(2-deoxy-β-D-erythropentofuranosyl)-3,4 dihydro-8H-pyrimido [4,5-c][1,2]oxazine-7-one 5'triphosphate.

16. A kit according to claim 14, further comprising wherein the means for replicating the template sequence comprises means for performing the polymerase chain reaction.

17. A kit according to claim 14, further comprising the four normal deoxynucleotides.

18. A kit according to claim 14, further comprising at least one member of the group consisting of 2'-deoxy-8-hydroxyguanosine 5'-triphosphate, 2-amino-9-(2-deoxy-β-D-erythropentofuranosyl)-6-methoxyaminopurine 5'-triphosphate, and O²-ethylthymidine triphosphate.

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5. The method of claims 1-4, wherein the MEK1 inhibitor is administered to a subject who has had an ischemic stroke.

6. The method of claims 1-4, wherein the MEK1 inhibitor is administered prophylactically to a subject at risk of having an ischemic stroke.

7. A pharmaceutical composition comprising

a MEK1 inhibitor, and

a non-MEK1 inhibitor anti-stroke agent, together in an amount effective for treating an ischemic condition.

8. A kit comprising a package housing

a first container containing a MEK1 inhibitor, and

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instructions for using the MEK1 inhibitor in the treatment of an ischemic condition.

9. The kit of claim 8, further comprising

a second container containing a non-MEK1 inhibitor anti-stroke agent.

10. A medical product comprising

an isolated organ in a perfusion fluid containing a MEK1 inhibitor.

11. A medical product comprising an organ perfusion fluid containing a MEK1 inhibitor.

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provided greater resolving power (statistical power) allowing the determination that dieldrin reduces sex ratio above about 30 ppb, a value lower than previously suspected of being biologically significant to *Daphnia*.

A decrease in sex ratio can occur via several basic mechanisms during early development, including:

1) Higher male mortality, with female production remaining the same. However, it was observed that the total number of offspring was not affected by dieldrin exposure.

2) Increase in female production, with an increase in total fecundity. However, there was no evidence that exposure to dieldrin increased fecundity.

3) A change in embryonic developmental rate, e.g., faster female maturation or slower male maturation induced by dieldrin exposure. However, male and female neonates are released at the same time from the adult's brood chamber.

4) Suppression of feeding rate or food quality which would also reduce total fecundity.

5) Seasonality which would not affect sex ratio in a 6-day bioassay.

6) A shift in the developmental process that results in fewer males and more females, with no change in fecundity.

The results of the bioassay supports the interpretation that dieldrin is an exogenous agent that interferes with the action of natural hormones in the *Daphnia* embryo that are responsible for reproduction development, i.e., dieldrin shows endocrine disruption activity in *Daphnia*. This endocrine disruption activity has two implications, one for aquatic ecology and one for human health.

Aquatic Ecology. Chemicals that change *Daphnia* development or reproduction are clearly of ecological concern. *Daphnia* is an ecologically important algae-consumer and fish-food in lakes all over the world. In particular, a decrease in the number of males has the potential of reducing *Daphnia*'s ecological success over many generations, because sexual production is thought essential for preparing a population for changes in the environment. Any chemical that interferes with normal *Daphnia* ecology will also have indirect effects on water quality and fish production.

Evidence indicates that *Daphnia* reproduction has changed during the last century (Dodson et al., *Envir. Health Perspectives* 103 (Special Suppl. No. 4) 7-11 (1996). In Lake Mendota, Wisconsin, *Daphnia*, which produces up to 50% males in the late 1800s, currently produces less than 5% males. One possibility for this change is the introduction of endocrine disruptors in to the environment beginning in the 1940s.

There is interest in synergistic effects of chemical mixtures and environmental factors because mixtures of chemicals are the norm in aquatic habitats. In the case of dieldrin and endosulfan, there was no evidence for greater-than-additive (synergistic) or enhanced effect of the mixture of these two pesticides. The lack of synergism in *Daphnia* is consistent with results of other studies of these two chemicals at the molecular level (Ramamoorthy et al., *Endocrinology* 138:1520-1527 (1997).

Human Health. Dieldrin, a chemical known to be an endocrine disrupter in vertebrates, also affects development and reproduction in *Daphnia*. The result shows that the present *Daphnia* bioassay can be used as a rapid screen to detect chemicals of potential concern for human health. *Daphnia* can be useful as a whole-animal invertebrate "canary down the mine shaft" that can provide a useful screen for endocrine disruption for both environmental and human health.

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What is claimed is:

1. A bioassay for testing a sample for the presence of a chemical substance that interferes with endocrine function in an animal, comprising:

maintaining a test sample and a control medium under conditions to induce sexual reproduction in *Daphnia*, the test sample and control each containing an effective number of adult, oviparous *Daphnia* of a single clone for crowding; and

comparing endpoints that indicate a deviation from normal *Daphnia* sexual reproduction in the test sample and control to determine the presence or absence of the chemical substance;

whereby the presence of an endocrine disrupter substance is indicated by a variance between the test sample and the control of the sex ratio, the ratio of males:total offspring the number of resting eggs, the number of neonates with a morphological abnormality the number of neonates with a behavioral abnormality the nutritional status of the offspring, or a combination thereof.

2. The bioassay according to claim 1, wherein the endpoints include survivorship of adults and neonates, fecundity, and at least one of the following endpoints: number of male offspring, sex ratio of males:total offspring (neonates), number of resting eggs, number of offspring having a morphological abnormality, number of offspring having a behavioral abnormality, and nutritional status of offspring.

3. The bioassay according to claim 1, wherein the sample and the control are maintained at a temperature of about 17-25° C., and a light:dark photoperiod of about 6-9 hours light to about 18-15 hours dark.

4. The bioassay according to claim 1, further comprising changing the sample and the control on about day 3-4 of the assay period, and discarding the neonate *Daphnia* from the sample and the control.

5. The bioassay according to claim 2, wherein the endpoints are measured in multigenerations of the *Daphnia* clone.

6. The bioassay according to claim 1, further comprising: an initial step of determining the sublethal concentration of the test sample.

7. The bioassay according to claim 6, wherein the sublethal concentration of the test sample is determined by maintaining a control medium and a series of aqueous dilutions of the test sample under growth conditions to induce sexual reproduction in *Daphnia*, each of the dilutions and the control containing an effective number of adult, oviparous *Daphnia* of a single clone for crowding;

comparing fecundity and survivorship in the dilutions and the control to determine the dilution having the highest concentration of the test sample at which survivorship and fecundity are maintained at about the same level as the control; and

using said dilution of the test sample in the assay.

8. The bioassay according to claim 1, wherein the *Daphnia* clone produces about 5-70% males of the total offspring under the control conditions.

9. The bioassay according to claim 2, wherein the morphological abnormality is identified by reduced or absent terminal setae on the second antennae, a forward curved tail spine, reduced swimming ability, or a combination thereof.

10. The bioassay according to claim 2, wherein the behavioral abnormality is identified by abnormal swimming or abnormal motility.

11. The bioassay according to claim 1, further comprising: conducting an assay to identify the chemical substance in the test sample.

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12. The bioassay according to claim 1, wherein the chemical substance is an herbicide, insecticide, fungicide, xenobiotic, plasticizer, phytohormone, phytoestrogen, organic solvent, or a combination thereof.

13. The bioassay according to claim 12, wherein the chemical substance is selected from the group consisting of estradiol, diethylstilbestrol, kepone, dichlorodiphenyltrichloroethane, dichlorodiphenyldichloroethane, 1-hydroxychlorodene, chlordane, zearalenone, coumestrol, nonylphenol, butylphenol, pentylphenol, isopentylphenol, polychlorinated biphenyl, chlorpyrifos, pentachlorophenol, atrazine, carbaryl, endosulfan, ethanol, and derivatives thereof.

14. The bioassay according to claim 1, wherein the substance is atrazine.

15. A bioassay for determining the sublethal level of toxicity of a substance, comprising:

maintaining a control medium and a series of dilutions of the substance in an aqueous medium under growth conditions to induce sexual reproduction in Daphnia, each of the dilutions and the control containing an effective number of adult, oviparous Daphnia of a single clone for crowding;

determining the sublethal level of toxicity of the substance by comparing fecundity and survivorship of Daphnia in the dilutions and the control to identify the dilution having the highest concentration of the substance at which survivorship and fecundity are at about the same level as the control.

16. The bioassay according to claim 15, further comprising:

comparing one or more endpoints that indicate a deviation from normal Daphnia sexual reproduction in the test sample dilution having the highest sublethal concentration of the substance.

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17. The bioassay according to claim 15, wherein the endpoints include the number of male offspring, number of female offspring, sex ratio of males: total offspring, number of resting eggs, and number of offspring displaying a morphological abnormality, number of offspring displaying a behavioral abnormality, and nutritional status of offspring.

18. A bioassay for determining the presence of a toxic chemical substance in a sample, comprising:

maintaining a control medium and the sample in an aqueous medium under growth conditions to induce sexual reproduction in Daphnia, each of the control and the sample containing an effective number of adult, oviparous Daphnia of a single clone for crowding; and comparing fecundity and survivorship of the Daphnia in the sample and the control;

wherein the presence of a toxic substance in the sample is determined by a lower level of fecundity, and survivorship of the Daphnia in the sample compared to the control.

19. A kit for use in conducting a Daphnia reproductive bioassay on an aqueous sample to detect a substance that interferes with endocrine function in an animal, comprising, in association and separately packaged:

a culture of a clone of Daphnia capable of producing at least about 5-70% males of the total offspring under control conditions; instructions for conducting the Daphnia bioassay according to claim 1; a data scoring sheet; and an algal food source for the Daphnia.

20. The kit according to claim 19, further comprising:

a container for the sample; a container for the control; a container for observing the Daphnia; a device for manipulating the Daphnia; a growth medium for culturing the Daphnia and the algae; or any combination thereof.

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What is claimed is:

1. A method of isolating a recombinant adeno-associated virus, comprising applying a sample containing recombinant adeno-associated virus to an iodixanol gradient, and collecting said recombinant adeno-associated virus from said gradient.
2. The method of claim 1, wherein said iodixanol gradient is a discontinuous gradient.
3. The method of claim 2, wherein said iodixanol gradient comprises an about 15% iodixanol step, an about 25% iodixanol step, an about 40% iodixanol step, and an about 60% iodixanol step.
4. The method of claim 3, wherein said recombinant adeno-associated virus is collected from said 40% iodixanol step.
5. The method of claim 3, wherein said 15% iodixanol step further comprises about 1 M NaCl.
6. The method of claim 1, wherein said iodixanol gradient is subjected to centrifugation after applying said sample.
7. The method of claim 1, further comprising contacting said recombinant adeno-associated virus with a matrix comprising heparin, under conditions effective to permit binding of said virus to said matrix, removing non-bound species from said matrix, and eluting said virus from said matrix.
8. The method of claim 7, wherein said matrix comprises heparin agarose type I or heparin agarose type II-S.
9. The method of claim 7, wherein said matrix is comprised within an HPLC column.
10. The method of claim 7, wherein said virus is eluted from said matrix with a solution comprising about 1 M NaCl.
11. The method of claim 1, further comprising contacting said recombinant adeno-associated virus with a hydrophobic matrix, under conditions effective to permit interaction of hydrophobic species with said hydrophobic matrix, and collecting non-interacting virus from said hydrophobic matrix.
12. The method of claim 11, wherein said hydrophobic matrix comprises phenyl groups.
13. The method of claim 12, wherein said hydrophobic matrix is phenyl-sepharose.
14. The method of claim 1, further comprising applying said recombinant adeno-associated virus to a cesium chloride equilibrium density gradient, and collecting said recombinant adeno-associated virus from said gradient.
15. The method of claim 1, further comprising contacting said recombinant adeno-associated virus with at least a first ion exchange chromatography medium, under conditions effective to permit interaction of said virus with said medium, removing non-interacting species from said medium, and eluting said virus from said medium.
16. The method of claim 1, wherein said sample further comprises a virus.
17. The method of claim 16, wherein said sample further comprises an adenovirus.
18. The method of claim 1, wherein said sample further comprises at least a first polypeptide or protein.
19. The method of claim 1, wherein said sample further comprises a cell extract or a growth medium.
20. A method of isolating a recombinant adeno-associated virus, comprising the steps of:
 - a) centrifuging a sample containing recombinant adeno-associated virus through an iodixanol gradient;
 - b) collecting from said iodixanol gradient at least a first fraction comprising said recombinant adeno-associated virus;

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- c) contacting said at least a first fraction comprising said recombinant adeno-associated virus with a matrix comprising heparin, under conditions effective to permit binding of said virus to said matrix;
- d) removing non-bound species from said matrix; and
- e) eluting said virus from said matrix.
21. A method of isolating a recombinant adeno-associated virus, comprising the steps of:
 - a) centrifuging a sample containing recombinant adeno-associated virus through an iodixanol gradient;
 - b) collecting from said iodixanol gradient at least a first fraction comprising said recombinant adeno-associated virus;
 - c) contacting said at least a first fraction comprising said recombinant adeno-associated virus with a matrix comprising heparin, under conditions effective to permit binding of said virus to said matrix;
 - d) removing non-bound species from said matrix;
 - e) eluting said virus from said matrix;
 - f) contacting the eluted virus with a hydrophobic matrix, under conditions effective to permit interaction of hydrophobic species with said hydrophobic matrix; and
 - g) collecting the non-interacting virus from said hydrophobic matrix.
22. A method for reducing or eliminating adenovirus from a recombinant adeno-associated virus composition contaminated with adenovirus, comprising applying a sample containing recombinant adeno-associated virus and adenovirus to an iodixanol gradient, and collecting from said gradient at least a first fraction comprising said recombinant adeno-associated virus.
23. A method of producing a recombinant adeno-associated virus having a particle-to-infectivity ratio of less than about 100 to 1, comprising the steps of:
 - a) centrifuging a sample containing recombinant adeno-associated virus through an iodixanol gradient;
 - b) collecting from said iodixanol gradient at least a first fraction comprising said recombinant adeno-associated virus;
 - c) contacting said at least a first fraction comprising said recombinant adeno-associated virus with a matrix comprising heparin, under conditions effective to permit binding of said virus to said matrix;
 - d) removing non-bound species from said matrix; and
 - e) eluting said virus from said matrix.
24. A kit comprising, in a suitable container, iodixanol, a matrix comprising heparin and instructions for isolating recombinant adeno-associated virus.
25. The kit of claim 24, wherein said iodixanol is formulated as an iodixanol gradient.
26. The kit of claim 24, wherein said matrix comprises heparin agarose type I or heparin agarose type II-S.
27. The kit of claim 24, further comprising a hydrophobic matrix.
28. The kit of claim 27, wherein said hydrophobic matrix comprises phenyl groups.
29. The kit of claim 28, wherein said hydrophobic matrix is phenyl-sepharose.

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- We claim:
1. A method for in vitro labeling of abnormal cervical cells with an acid phosphatase enzyme reaction product that increases visibility of said cells on Pap smears, comprising the steps of:
 - a) scrapping cervical epithelium by an abrasive device,
 - b) obtaining an analytical specimen composed of cervical cells from more than one layer of cervical epithelium,
 - c) spreading said specimen over a microscopic slide to form a smear of separated cells,
 - d) exposing said smears to air fixation before transporting to a laboratory,
 - e) exposing said smears to fixation with a solution containing at least citrate acetone or formaldehyde at room temperature,
 - f) incubating said smears, after fixation, with an incubation mixture containing at least a diazonium salt and a naphthol phosphate in water, for time, temperature and pH, optimal for said acid phosphatase reaction to complete,
 - g) exposing said smears, after incubation, to staining with a modified Papanicolaou staining method,
 - h) mounting said smears after completion of said Papanicolaou staining, and
 - i) investigating said smears, after mounting, under a microscope using new criteria for classification of smears stained by the CAP-PAP test,
 whereby said method allow a human observer, or a machine, to diagnose said specimens into categories of negative, nonnegative or positive for cervical dysplasia and/or cervical cancer.
 2. A kit which comprises a carrying box comprising:
 - a) labeled bottles containing reagents for use in a method for in vitro labeling of abnormal cervical cells with an acid phosphatase enzyme reaction,
 - b) control slides, comprising
 - (1) microscopic slides with HeLa cells smeared on, and
 - (2) microscopic slides stained with HeLa cells, and
 - c) written instructions for using said reagents to perform said method on analytical specimens obtained from healthy women or patients, and
 - d) written instructions for using said HeLa smears for quality control and/or quality assurance of said method.
 3. An assembly of instruments comprising at least
 - a) an automatic mechanical device to combine all steps of a method for in vitro labeling of abnormal cervical cells with an acid phosphatase enzyme reaction into a continuous stepwise procedure, comprising:
 - i) a train to carry unstained smears throughout a series of stations, and
 - ii) a series of stations providing technical facility for performance of every step of the staining procedure, and
 - iii) a motor to move said train with smears throughout said stations and said staining procedure,
 whereby said devices are combined into an automatic staining device for processing said marker and mak-

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| Contents | Volume | Dimensions-Flat (L x W) | Assembled (L x W x H) |
|-----------------------|----------------|-------------------------|-----------------------|
| 2 Paper Pads | | | |
| 3 Small Cartons | 1.5 cubic feet | 26" x 29" | 16" x 12½" x 12½" |
| 10 lbs. Packing Paper | | | |
| 3 Medium Cartons | 3.1 cubic feet | 35" x 36" | 18" x 18" x 16" |
| 3 Large Cartons | 4.5 cubic feet | 36½" x 42½" | 24" x 18" x 18" |
| 1 Dish Carton | 5.2 cubic feet | 47" x 36½" | 18" x 18" x 28" |
| 1 Mirror Box (2 pcs.) | | | |
| Package Container | | | |

KITCHEN PACKING KIT

| Contents | Volume | Dimensions-Flat (L x W) | Assembled (L x W x H) |
|-----------------------|----------------|-------------------------|-----------------------|
| 1 Packing Guide | N/A | N/A | N/A |
| 1 Marker | | | |
| 2 Tape Rolls | | | |
| 2 Small Cartons | 1.5 cubic feet | 26" x 29" | 16" x 12½" x 12½" |
| 30 lbs. Packing Paper | | | |
| 3 Medium Cartons | 3.1 cubic feet | 35" x 36" | 18" x 18" x 16" |
| 3 Large Cartons | 4.5 cubic feet | 36½" x 42½" | 24" x 18" x 18" |
| 5 Dish Cartons | 5.2 cubic feet | 47" x 36½" | 18" x 18" x 28" |
| Package Container | | | |

LIVING ROOM PACKING KIT

| Contents | Volume | Dimensions-Flat (L x W) | Assembled (L x W x H) |
|-----------------------|----------------|-------------------------|-----------------------|
| 1 Packing Guide | N/A | N/A | N/A |
| 1 Marker | | | |
| 2 Tape Rolls | | | |
| 2 Paper Pads | | | |
| 4 Small Cartons | 1.5 cubic feet | 26" x 29" | 16" x 12½" x 12½" |
| 20 lbs. Packing Paper | | | |
| 5 Medium Cartons | 3.1 cubic feet | 35" x 36" | 18" x 18" x 16" |
| 4 Large Cartons | 4.5 cubic feet | 36½" x 42½" | 24" x 18" x 18" |
| 1 Dish Carton | 5.2 cubic feet | 47" x 36½" | 18" x 18" x 28" |
| 1 Mirror Box (2 pcs.) | | | |
| Package Container | | | |

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What is claimed is:

1. A packing kit adapted for use in packing items for moving transport, comprising in combination:

- a. at least one packing carton for packing items;
- b. mastic means for appropriately closing and securing said carton;
- c. packing material to maintain positions of items in said carton;
- d. inscribing means to label said items in the carton or said carton;
- e. instructions on use of contents of said packing kit to effect the professional packing of items by use of other elements of the kit.

2. The packing kit of claim 1 wherein said packing carton is selected from the group of corrugated cardboard, paper, or plastic.

3. The packing kit of claim 1 wherein said mastic means is selected from the group consisting of self adhesive tapes, gummed or water moistened tape, or glue.

4. The packing kit of claim 1 wherein said packing material is selected from the group consisting of paper pads, newsprint, and plastic cushioning.

5. The packing kit of claim 1 wherein said inscribing means is selected from the group consisting of indelible markers, pens, pencils, crayons, pre-printed labels or label makers.

6. The packing kit of claim 1 wherein said at least one packing carton is selected from the group consisting of at least one carton measuring 1.5 cubic feet in volume, at least one carton measuring 3.1 cubic feet in volume, at least one carton measuring 4.5 cubic feet in volume, at least one cartons measuring 5.2 cubic feet in volume, at least one two-piece mirror container.

7. The packing kit of claim 1 wherein said packaging for the kit is packaged by banding or other container.

8. A general packing kit adapted for use in packing items for moving transport, comprising in combination:

an instruction guide, at least one inscribing means, at least 2 units of mastic means, at least 2 paper pads, at least 4 cartons measuring 1.5 cubic feet in volume, at least 20 lbs. of newsprint, at least 4 cartons measuring 3.1 cubic feet in volume, at least 4 cartons measuring 4.5 cubic feet in volume, at least 2 cartons measuring 5.2 cubic feet in volume, at least one two-piece mirror container and at least one package container.

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What is claimed is:

1. A method of treating vascular stenosis in a mammal in need of same, comprising administering an effective amount of hepatocyte growth factor ("HGF") to the mammal.

2. The method of claim 1 wherein said HGF is human HGF.

3. The method of claim 1 wherein said HGF is administered intraarterially.

4. The method of claim 3 wherein said HGF is further administered intravenously.

5. An article of manufacture, comprising:

a container;

a label on said container; and

a composition contained within said container; wherein the composition is an effective amount for treating vascular stenosis, the label on said container indicates that the composition can be used for treating vascular stenosis, and the active agent in said composition comprises HGF.

6. The article of manufacture of claim 5 further comprising instructions for administering the HGF to a mammal.

7. A kit, comprising:

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a first container, a label on said container, and

a composition contained within said container; wherein the composition is an effective amount for treating vascular stenosis, the label on said container indicates that the composition can be used for treating vascular stenosis, and the active agent in said composition comprises HGF;

a second container comprising a pharmaceutically-acceptable buffer; and

instructions for using the HGF to treat vascular stenosis.

8. A method of treating vascular stenosis in a mammal due to trauma or insult to blood vessel walls of said mammal, comprising administering to a mammal in need thereof an amount of HGF effective to enhance endothelial cell re-surfacing of said blood vessel, as compared to the pace of re-surfacing in an absence of administered HGF.

9. The method of claim 8, wherein said vascular insult or trauma is due to vascular surgery.

10. The method of claim 8, wherein said vascular insult or trauma is due to angioplasty.

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to a point, e.g., beneath the rib cage. The cannula 10 may be implanted and connected to the port 20, in a manner analogous to that described above for the bladder connection. The port may then be accessed using an access tube and drained, either by gravity or using a suitable vacuum-applying system.

Referring now to FIG. 10, drainage of ascites which may accumulate in an abdominal region AR of a patient is illustrated in FIG. 10. A cannula 10 is located with its distal, collection end within the region of the abdomen where ascites fluid is collecting. Port 20 is implanted, usually just outside the region where the ascites is collecting. The fluid may be collected through the port, either by gravity or by application of a vacuum, as with the previously described embodiments.

As a final exemplary embodiment, the cannula 10 and port 20 may be connected to drain cerebrospinal fluid (CSF) from a region within a patient's meninges M, as illustrated in FIG. 11. The meninges is a thin membrane which encases the brain and the vertebral column and includes the subarachnoid space, the ventricles, and the brain interstitial spaces. In patients suffering from hydrocephalus, excess CSF may accumulate within the meninges and must be drained in order to reduce intracranial pressures to a safe level. Drainage is effected by implanting the distal, collection end of the cannula within any convenient region within the meninges, typically within a brain ventricle. The cannula 10 is then passed subcutaneously to a region in the patient's torso, typically in the chest region as shown in FIG. 11. The implanted port 20 may then be accessed using an access tube and CSF drained, either by gravity or by applying a vacuum, as generally described for the previous embodiments.

While the above is a complete description of the preferred embodiments of the invention, various alternatives, modifications, and equivalents may be used. Therefore, the above description should not be taken as limiting the scope of the invention which is defined by the appended claims.

What is claimed is:

1. A method for removing endogenous fluids from a patient, said method comprising:
percutaneously introducing an access tube to an implanted port connected to a site of endogenous fluid retention, wherein the endogenous fluid has accumulated as a result of an abnormal condition; and

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removing at least 50% by volume of the endogenous fluid which has accumulated at the site through a fluid conductive path defined by the access tube and the port.

2. A method as in claim 1, wherein removing endogenous fluid comprises applying a vacuum through the access tube.

3. A method as in claim 1, wherein the site is selected from the group consisting of the pleural space, the bladder, the abdomen, and the meninges.

4. A method as in claim 3, wherein the fluid is selected from the group consisting of pleural fluid, urine, ascites, and cerebrospinal fluid.

5. A method as in claim 1, wherein the port is normally closed and opens in response to introduction of the access tube.

6. A method as in claim 1, wherein the access tube is introduced in a direction which is substantially perpendicular to the patient's skin at the point where the tube is introduced.

7. A method as in claim 1, further comprising disinfecting the port prior to or after introducing the access tube.

8. A method as in claim 7, wherein disinfecting comprises percutaneously introducing a disinfectant medium to a location in or around the port while the port remains closed.

9. A kit for removing endogenous fluids from a patient, said kit comprising:

an access tube adapted for percutaneous introduction to an implanted port; and

- instructions for use setting forth a method for removing endogenous fluids from a patient in accordance with the following steps:

percutaneously introducing an access tube to an implanted port connected to a site of endogenous fluid retention, wherein the endogenous fluid has accumulated as a result of an abnormal condition; and

removing at least 50% by volume of the endogenous fluid which has accumulated at the site through a fluid conductive path defined by the access tube and the port.

10. A kit as in claim 9, further comprising an aspirator which includes the access tube.

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strip during the second set of determinations is expressed as a percent of the peak tension developed during the first concentration-effect determination. Then, for each test substance the resultant data are analyzed for treatment-related differences by one-way analysis of variance (ANOVA). Since only one concentration of test substance is studied in each strip of bladder, the procedures of Arunlakshana and Schild (1959) are used in modified form to estimate the pA₂ and slope of the Schild regression.

First, the concentrations of agonist producing a half-maximal response (the EC₅₀) is estimated for each strip from the second set of concentration-effect data. The EC₅₀ is obtained from linear regression lines fit to the logarithm of the concentration of drug and the responses bracketing the half maximum level of response. For each drug-treated strip, a "concentration ratio" (CR) is calculated as the ratio of the EC₅₀ of the treated tissue divided by the EC₅₀ of the untreated tissue. For each experiment where two or more strips are exposed to the same test substance but at different concentrations, the logarithm of this ratio minus one [i. e., log (CR-1)] is plotted against the logarithm of the concentration of antagonist to which the strip had been exposed to produce "Schild plots". A regression analysis relating log (CR-1) to the logarithm of the concentration of the antagonist is employed to estimate the pA₂ and the slope of the regression line.

Finally, experiments are grouped by test substance and the mean \pm S.E. of the pA₂ and slope are calculated. The 95% confidence limits (CL) for the slope are estimated from its S.E. using standard methods. For experiments in which only one strip is exposed to a given test substance, a pKD is calculated as (concentration of antagonist)/(CR-1) and the negative logarithm of the KD is then pooled with the pA₂ values to yield an expanded set of pA₂ values.

The embodiments of the present invention described above are intended to be merely exemplary and those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. All such equivalents are considered to be within the scope of the present invention and are covered by the following claims.

The contents of all references described herein are hereby incorporated by reference.

Other embodiments are within the following claims.

What is claimed is:

1. A method for treating urinary incontinence, comprising administering to a subject in need thereof a therapeutically effective amount of enantiomerically enriched (S)-procyclidine, or a pharmaceutically acceptable salt thereof.

2. The method as recited in claim 1, wherein the enantiomerically enriched (S)-procyclidine, or a pharmaceutically acceptable salt thereof, is administered as a pharmaceutical composition comprising said enantiomerically enriched (S)-procyclidine, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

3. The method as recited in claim 2, wherein said pharmaceutical composition is administered by oral, parenteral, transdermal, rectal, or vaginal administration.

4. The method as recited in claim 2, wherein said pharmaceutical composition is administered by injection or intravesical perfusion.

5. The method as recited in claim 1, wherein a daily amount of (S)-procyclidine administered in the preparation is about 0.25 mg to about 500 mg.

6. The method as recited in claim 5, wherein the daily amount of (S)-procyclidine administered in the preparation is about 1 mg to about 100 mg.

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7. The method as recited in claim 2, wherein (S)-procyclidine comprises greater than 50% by weight of the total procyclidine in said pharmaceutical composition.

8. The method as recited in claim 7, wherein (S)-procyclidine comprises at least 80% by weight of the total procyclidine in said pharmaceutical composition.

9. The method as recited in claim 1, wherein (S)-procyclidine is administered as a pharmaceutical composition comprising substantially enantiomerically enriched (S)-procyclidine, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

10. The method as recited in claim 9, wherein (S)-procyclidine comprises at least 85% by weight of the total procyclidine in said pharmaceutical composition.

11. The method as recited in claim 10, wherein (S)-procyclidine comprises at least 99% by weight of the total procyclidine in said pharmaceutical composition.

12. The method as recited in claim 1, wherein the subject is treated for stress incontinence, urge incontinence, post-prostatectomy incontinence or enuresis.

13. A method for treating bladder detrusor muscle instability, said method comprising administering to a subject in need thereof a therapeutically effective amount of enantiomerically enriched (S)-procyclidine, or a pharmaceutically acceptable salt thereof.

14. The method as recited in claim 13, wherein said enantiomerically enriched (S)-procyclidine, or a pharmaceutically acceptable salt thereof, is substantially free of (R)-procyclidine.

15. A pharmaceutical unit dosage form, which comprises enantiomerically enriched (S)-procyclidine, or a pharmaceutically acceptable salt thereof, and a pharmaceutical carrier, wherein said pharmaceutical unit dosage form is a tablet.

16. The pharmaceutical unit dosage form as recited in claim 15, wherein said enantiomerically enriched (S)-procyclidine is present in an amount of about 0.25 mg to about 250 mg.

17. The pharmaceutical unit dosage form as recited in claim 16, wherein said enantiomerically enriched (S)-procyclidine is present in an amount of about 1 mg to about 50 mg.

18. A pharmaceutical unit dosage form which comprises substantially enantiomerically enriched (S)-procyclidine, or a pharmaceutically acceptable salt thereof, and a pharmaceutical carrier, wherein said pharmaceutical unit dosage form is a tablet.

19. A pharmaceutical unit dosage form, which comprises enantiomerically enriched (S)-procyclidine, or a pharmaceutically acceptable salt thereof, and a pharmaceutical carrier, wherein said pharmaceutical unit dosage form is a soft elastic gelatin capsule.

20. The pharmaceutical unit dosage form as recited in claim 19, wherein said enantiomerically enriched (S)-procyclidine is present in an amount of about 0.25 mg to about 250 mg.

21. The pharmaceutical unit dosage form as recited in claim 20, wherein said enantiomerically enriched (S)-procyclidine is present in an amount of about 1 mg to about 50 mg.

22. A pharmaceutical unit dosage form which comprises substantially enantiomerically enriched (S)-procyclidine, or a pharmaceutically acceptable salt thereof, and a pharmaceutical carrier, wherein said pharmaceutical unit dosage form is a soft elastic gelatin capsule.

23. A kit for treating urinary incontinence, wherein said kit comprises a pharmaceutical composition comprising enantiomerically enriched (S)-procyclidine, or a pharmaceutically acceptable salt thereof, and a pharmaceutically

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✓ acceptable carrier, and instructions for administering enantiomerically enriched (S)-procyclidine for the treatment of urinary incontinence while reducing or eliminating concomitant liability of adverse effect.

24. The kit as recited in claim 23, wherein said adverse effect is one or more of drowsiness, epistaxis, xerostomia, mydriasis, cycloplegia, cardiovascular tachycardia, cardiovascular palpitations, increased ocular pressure, nausea, constipation, decreased sweating, impotence, or unwanted dermal manifestations.

25. A kit for treating urinary incontinence, wherein said kit comprises a pharmaceutical composition comprising substantially enantiomerically enriched (S)-procyclidine, or a pharmaceutically acceptable salt thereof, and a pharma-

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ceutically acceptable carrier, and instructions for administering substantially enantiomerically enriched (S)-procyclidine for the treatment of urinary incontinence while reducing or eliminating concomitant liability of adverse effects.

26. The kit as recited in claim 25 wherein said adverse effect is one or more of drowsiness, epistaxis, xerostomia, mydriasis, cycloplegia, cardiovascular tachycardia, cardiovascular palpitations, increased ocular pressure, nausea, constipation, decreased sweating, impotence, or unwanted dermal manifestations.

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66. The formulation of claim 55, wherein the Type IV phosphodiesterase inhibitor is a quinazolinone.

67. The formulation of claim 66, wherein the quinazolinone is selected from the group consisting of nitraquazone, CP-77059 and RS-25344.

68. The formulation of claim 55, wherein the Type IV phosphodiesterase inhibitor is a xanthine derivative.

69. The formulation of claim 68, wherein the xanthine derivative is selected from the group consisting of denbufylline, arofyline and BRL 61063.

70. The formulation of claim 55, wherein the Type IV phosphodiesterase inhibitor is a phenyl ethyl pyridine.

71. The formulation of claim 70, wherein the phenyl ethyl pyridine is CDP 840.

72. The formulation of claim 55, wherein the Type IV phosphodiesterase inhibitor is a tetrahydropyrimidinone.

73. The formulation of claim 72, wherein the tetrahydropyrimidinone is atizoram.

74. The formulation of claim 55, wherein the Type IV phosphodiesterase inhibitor is a diazepine derivative.

75. The formulation of claim 74, wherein the diazepine derivative is CI 1018.

76. The formulation of claim 55, wherein the Type IV phosphodiesterase inhibitor is an oxime carbamate.

77. The formulation of claim 76, wherein the oxime carbamate is flaminast.

78. The formulation of claim 55, wherein the Type IV phosphodiesterase inhibitor is a naphthyridinone.

79. The formulation of claim 78, wherein the naphthyridinone is RS 17597.

80. The formulation of claim 55, wherein the Type IV phosphodiesterase inhibitor is a benzofuran.

81. The formulation of claim 80, wherein the benzofuran is selected from the group consisting of 2-butyl-7-methoxy-benzofuran-4-carboxylic acid (3-5-dichloropyridin-4-yl)-amide, 2-benzyl-7-methoxy-benzofuran-4-carboxylic acid (3-5-dichloropyridin-4-yl)-amide, 7-methoxy-2-phenethyl-benzofuran-4-carboxylic acid (3-5-dichloropyridin-4-yl)-amide, 5-(2-butyl-7-methoxy-benzofuran-4-yl)-tetrahydropyrimidin-2-one, phenyldihydrobenzofuranes, 4-substituted benzofuranes and substituted furans.

82. The formulation of claim 55, wherein the Type IV phosphodiesterase inhibitor is a naphthalene derivative.

83. The formulation of claim 82, wherein the naphthalene derivative is T 440.

84. The formulation of claim 55, wherein the Type IV phosphodiesterase inhibitor is a purine derivative.

85. The formulation of claim 84, wherein the purine derivative is V-12294A.

86. The formulation of claim 55, wherein the Type IV phosphodiesterase inhibitor is a cyclohexane carboxylic acid.

87. The formulation of claim 86, wherein the cyclohexane carboxylic acid is ariflo.

88. The formulation of claim 55, wherein the Type IV phosphodiesterase inhibitor is a benzamide.

89. The formulation of claim 88, wherein the benzamide is piclamilast.

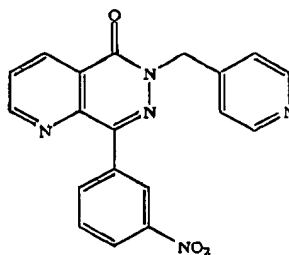
90. The formulation of claim 55, wherein the Type IV phosphodiesterase inhibitor is a benzothiophene.

91. The formulation of claim 90, wherein the benzothiophene is thienelast.

92. The formulation of claim 55, wherein the Type IV phosphodiesterase inhibitor is a pyridopyridazinone.

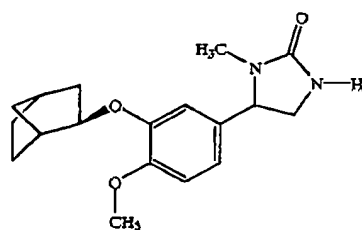
93. The formulation of claim 92, wherein the pyridopyridazinone has the structural formula

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94. The formulation of claim 55, wherein the Type IV phosphodiesterase inhibitor is an imidazolidinone.

95. The formulation of claim 94, wherein the imidazolidinone has the structural formula



96. The formulation of claim 55, further including an additional active agent.

97. The formulation of claim 96, wherein the additional active agent is an additional phosphodiesterase inhibitor.

98. The formulation of claim 97, wherein the additional active agent is an additional Type IV phosphodiesterase inhibitor.

99. The formulation of claim 97, wherein the additional active agent is a Type III phosphodiesterase inhibitor.

100. The formulation of claim 97, wherein the additional active agent is a Type V phosphodiesterase inhibitor.

101. The formulation of claim 96, wherein the additional active agent is selected from the group consisting of nitrovasodilators, alpha receptor blocking agents, ergot alkaloids, naturally occurring, semisynthetic and synthetic prostaglandins; vasoactive intestinal peptide; and combinations thereof.

102. The formulation of claim 101, wherein the additional active agent is a prostaglandin.

103. The formulation of claim 102, wherein the additional active agent is PGE₁.

104. A kit for treating erectile dysfunction, comprising: a pharmaceutical formulation containing a Type IV phosphodiesterase inhibitor or a pharmaceutically acceptable salt, ester, amide or prodrug thereof; a means for locally administering the formulation; a container for housing the formulation and drug delivery means; and instructions for using the drug delivery means to administer the formulation within the context of a dosing regimen effective to treat erectile dysfunction.

105. The kit of claim 104, wherein the formulation comprises a urethral dosage form, and the means for administering the agent is a transurethral drug delivery device.

106. The kit of claim 105, further including a flexible, adjustable venous flow control (VFC) device and instructions for using the VFC device.

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61. A method as in claim 55, wherein the port comprises an aperture and the locating step comprises manually aligning the cannula with a line from the skin entry point of the access tract to the aperture on the port.

62. A method as in claim 61, wherein the aperture has dimensions which correspond to the cannula.

63. A method as in claim 61, wherein the locating step further comprise annularly feeling the port to determine the position of the aperture.

64. A method as in claim 55, wherein the introducing step comprises orienting the cannula generally vertically with respect to the skin surface.

65. A method as in claim 55, wherein the cannula is introduced through a skin layer having a thickness in the range from 3 mm to 20 mm.

66. A method as in claim 55, wherein the port has an internal valve that inhibits bleed back into the tissue tract after the cannula is withdrawn.

67. A method as in claim 55, wherein the cannula comprises a needle.

68. A method as in claim 55, wherein the cannula comprises a blunt tube.

69. A method as in claim 68, wherein the cannula further comprises a stylet removably received in the blunt tube.

70. A method for forming a percutaneous access tract to an implanted port, said method comprising:

percutaneous introducing a cannula to define an access tract having a skin entry point and extending to the port, wherein the cannula has a bore size of at least 1.16 mm and dilates the access tract as it is introduced therethrough; and

repeatedly accessing the port with a cannula through the same access tract at intervals and over a time period sufficient to cause scar tissue formation over the access tract.

71. A method as in claim 70, wherein the accessing step comprises introducing a cannula at intervals of at least twice a week for a period of at least three months.

72. A method as in claim 70, wherein the accessing step comprises introducing a cannula at intervals of at least twice a day for a period of at least three months.

73. A method as in claim 70, wherein the introducing step comprises orienting the cannula generally vertically with respect to the skin surface.

74. A method as in claim 70, wherein the cannula is introduced through a skin layer having a thickness in the range from 3 mm to 20 mm.

75. A method as in claim 70, wherein the port has an internal valve that inhibits bleed back into the tissue tract after the cannula is withdrawn.

76. A method as in claim 70, wherein the cannula comprises a needle.

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77. A method as in claim 70, wherein the cannula comprises a blunt tube.

78. A method as in claim 77, wherein the cannula further comprises a stylet removably received in the blunt tube.

79. A kit comprising:

a cannula;

instructions for use setting forth a method according to claim 60; and

a package containing the cannula and the instructions for use.

80. A kit as in claim 79, further comprising a catheter connected or connectable to the cannula.

81. A kit comprising:

a cannula;

instructions for use setting forth a method according to claim 70; and

a package containing the cannula and the instructions for use.

82. A kit as in claim 81, further comprising a catheter connected or connectable to the cannula.

83. A method for percutaneously accessing an implanted port, said method comprising:

locating a preformed access tract from a skin entry point to the port; and

percutaneously introducing a cannula comprising a blunt tube having a stylet removably received therein through the preformed access tract to establish a flow path through the cannula to the port.

84. A method as in claim 83, wherein the port comprises an aperture and the locating step comprises manually aligning the cannula with a line from the skin entry point of the access tract to the aperture on the port.

85. A method as in claim 84, wherein the aperture has dimensions which correspond to the cannula.

86. A method as in claim 84, wherein the locating step further comprise annularly feeling the port to determine the position of the aperture.

87. A method as in claim 83, wherein the introducing step comprises orienting the cannula generally vertically with respect to the skin surface.

88. A method as in claim 83, wherein the cannula is introduced through a skin layer having a thickness in the range from 3 mm to 20 mm.

89. A method as in claim 83, wherein the port has an internal valve that inhibits bleed back into the tissue tract after the cannula is withdrawn.

90. A method as in claim 83, wherein the cannula dilates the access tract as it is introduced therethrough.

91. A method as in claim 90, wherein the cannula has a bore size of at least 1.16 mm.

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The following examples are provided for a further understanding of the invention, however, the invention is not to be construed as being limited thereto.

COMPARATIVE EXAMPLE

The following instructions are taken directly from the instructions using Canon T-Shirt Transfers TR-101.

Items Required

Canon T-shirt Transfer paper TR-101

Canon Color Bubble Jet BJC-4000 series or BJC-600 series printer.

Cotton or cotton-poly blend garment or fabric. Light colors work best.

Household hand iron.

Pillow case.

Ironing Surface- use on formica or glass surface only. Do not use ironing board. Be sure that the ironing surface is smooth and free of any imperfections (scratches, dents, etc) as it will affect the transfer.

Printing the Transfer

Insert one transfer sheet into printer with the blank side face up. Do not insert more than one transfer sheet into printer at a time. If lead edge is curled, straighten before feeding.

Set the paper selection lever to the back position.

Use any application to create the image.

Before printing the image, make sure that "Media Options" setting in the printer driver has been set to "Back Print Film". This setting will print the image in reverse on the transfer media so that it will appear correctly when it is ironed on.

Preparing the Transfer

For best results cut away the unprinted portion of the transfer, coming as close to the printed area as possible.

If an unprinted portion of the transfer is applied to the fabric it will cause the fabric to become stiff.

Ironing Instructions

The following numbered steps correspond to the steps in the directions in Canon T-Shirt Transfers TR-101 (copyright 1995); CST-4051-002.

1. Pre-heat iron on "highest" setting for 8 minutes.

2. Place the pillowcase, with the seam overhanging the edge, on the ironing surface.

3. Fold the pillowcase in half, with the seam still hanging over the edge of the ironing surface.

4. Place the garment onto the wrinkle-free pillowcase centering the area of the garment which will receive the printed transfer over the pillowcase. Be sure garment is wrinkle-free.

5. Center transfer, printed side down onto the garment (making certain the entire transfer is over the pillowcase).

Before ironing, make a small fold in lower left corner of transfer. Only $\frac{1}{4}$ " fold is necessary. Do not fold beyond the edge of printed area underneath. This fold will provide a good grip from which to peel the transfer.

6. Place the iron over both edges of the transfer, beginning in the upper left corner. The hand iron will always be positioned point facing down for each step.

7. Starting from one edge, push iron slowly along the long side of the transfer for at least 15 seconds using firm body pressure while pressing down on iron. Be certain iron overlaps all edges of transfer. While pushing iron, count 1,000, 2,000, 3,000 up to 15,000. This will take approximately 15 seconds.

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8. Reposition iron over both edges of the transfer beginning in the lower left corner.

9. Starting from one edge, push iron slowly along long side of transfer for 15 seconds using firm body pressure while pressing down iron. Be certain iron overlaps all edges of transfer.

10. Repeat Steps 6 through 9.

11. Immediately reheat entire surface by making 10 complete circles with iron over the transfer, being absolutely certain that the flat bottom of the iron covers the entire surface of the transfer paying special attention to all edges and corners.

12. Immediately place iron aside, beginning with folded-in corner, peel transfer from fabric using firm, steady pressure. If transfer is difficult to peel from shirt, do not fight it. Simply reheat that section of transfer which does not release by making three light circles, covering all edges and corners with flat part of iron. Place iron down immediately and peel while hot.

13. Gently smooth the fabric with your hands and allow to cool for at least at 2 minutes.

INVENTIVE EXAMPLE 1

Repeat steps 1-12 of the comparative example. After step 12 or optionally after step 13, place a silicone sheet over the imaged fabric. Press the silicone sheet by hand ironing at the highest setting on the iron to drive the coating into the valleys of the fabric by repeating steps 6-9 of the Comparative Example (with the silicone sheet), except change the time from 15 to 30 seconds for each of steps 7 and 9.

INVENTIVE EXAMPLE 2

Referring to FIG. 2, the method of applying the image and non-image areas to a receptor element will be described. The imaging sheet 50 is prepared, exposed and developed to form an image as in Example 1 of U.S. Pat. No. 5,139,917. A receptor element (e.g., tee shirt 62) is laid flat as illustrated, on an appropriate support surface, and the front surface of the imaging sheet 50 is positioned on the tee-shirt. An iron 64 is run and pressed across the back 52A of the imaging sheet. The image and non-image areas are transferred to the tee-shirt and the support is removed and discarded. Place a silicone sheet over the imaged fabric. Press the silicone sheet by hand ironing at the highest setting on the iron to drive the coating into the valleys of the fabric by repeating steps 6-9 of the Comparative Example (with the silicone sheet), except change the time from 15 to 30 seconds for each of steps 7 and 9.

The contents of each of the above-mentioned U.S. patents, copending applications and provisional applications are herein incorporated by reference.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the present invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

What is claimed is:

1. A kit, comprising:

(a) at least one transfer material comprising a support sheet and a transfer coating thereon, said support sheet having a front and back surface, said transfer coating positioned on said front surface of said support sheet, said transfer coating capable of melting and adhering to a receptor element having valleys or pores on the surface thereof by hand ironing,

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- (b) a tack resistant and stick resistant overlying sheet, and
 (c) instructions for transferring an image from the transfer sheet to a receptor element using said transfer sheet, said instructions comprising the steps of:
- (i) hand ironing the rear surface of an imaged transfer material,
 - (ii) peeling away the support sheet,
 - (iii) placing a non-stick sheet over the imaged receptor element, and
 - (iv) pressing the non-stick sheet with a hand iron in order to press the transfer coating into the valleys of the receptor element.
2. The kit of claim 1, wherein said transfer coating is capable of transferring and adhering image and non-image areas from said front surface of said support upon the application of heat energy to the rear surface of the support, said transfer coating strips from said front surface of the support by liquefying and releasing from said support upon heated, said liquefied carrier providing adherence to a receptor element by flowing onto said receptor element and solidifying thereon, said adherence does not require an external adhesive layer.
3. The kit of claim 1, wherein said transfer material is a Cyclic transfer material.
4. The kit of claim 1, wherein said receptor element is a tee shirt.
5. The kit of claim 1, wherein the transfer material is an image-receptive heat transfer material having front and rear surfaces, which comprises:
- a flexible cellulosic nonwoven web base sheet having top and bottom surfaces,
 - an image-receptive melt-transfer film layer overlaying the top surface of said base sheet, which image-receptive melt-transfer film layer is comprised of a thermoplastic polymer selected from the group consisting of polyolefins, polyesters, and ethylene-vinyl acetate copolymers and which melts in the range of from about 65 to about 180 degrees Celsius, in which the exposed surface of said image-receptive melt-transfer layers has a smoothness value, independent of the smoothness of the base sheet, of at least about 10 cc/minute as measured by a Sheffield Smoothness Tester.
6. The kit of claim 1, wherein the transfer material is an image-receptive heat transfer material having front and rear surfaces, which comprises:
- a flexible cellulosic nonwoven web base sheet having top and bottom surfaces,
 - a melt extruded, melt-transfer film layer overlaying the top surface of said base sheet, which melt transfer film layer is comprised of a first thermoplastic polymer selected from the group consisting of polyolefins, polyesters, and ethylene-vinyl acetate copolymers, ethylene-methacrylic acid copolymers, and ethylene-acrylic acid copolymers, and which melts in the range of from about 65 to about 180 degrees Celsius, and
 - a melt-extruded, image receptive film layer overlaying said melt-transfer layer, which image-receptive film layer is comprised of a second thermoplastic polymer selected from the group consisting of polyolefins, polyesters, and ethylene-vinyl acetate copolymers and which melts in the range of from about 65 to about 180 degrees Celsius, in which the exposed surface of said image-receptive melt-transfer layers has a smoothness value, independent of the smoothness of the base sheet, of at least about 10 cc/minute as measured by a Sheffield Smoothness Tester.

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7. The kit of claim 1, wherein the transfer material is an image-receptive heat transfer material having front and rear surfaces, which comprises:
- a flexible cellulosic nonwoven web base sheet having top and bottom surfaces,
 - an image-receptive melt-transfer film layer overlaying the top surface of said base sheet, which image-receptive melt-transfer film layer comprises about 15 to about 80 percent by weight of a film-forming binder selected from the group consisting of ethylene-acrylic acid copolymers, polyolefins, and waxes and from about 85 to about 20 percent by weight of a powdered thermoplastic polymer selected from the group consisting of polyolefins, polyesters, polyamides, waxes, epoxy polymers, ethylene-acrylic acid copolymers, and ethylene-vinyl acetate copolymers, wherein each of said film-forming binder and said powdered thermoplastic polymer melts in the range of from about 65° C. to about 180 degrees Celsius and said powdered thermoplastic polymer consists of particles of about 2 to about 50 micrometers in diameter.
8. The kit of claim 1, wherein the transfer material is an image-receptive heat transfer material having front and rear surfaces, which comprises:
- a flexible cellulosic nonwoven web base sheet having top and bottom surfaces,
 - a melt transfer film layer overlaying the top surface of said base sheet, which melt transfer film layer comprises a film forming binder selected from the group consisting of ethylene-acrylic acid copolymers, polyolefins, and waxes and which melts in the range of from about 65 to about 180 degrees Celsius, and
 - an image-receptive film layer overlaying said melt-transfer film layer, which image-receptive film layer comprises about 15 to about 80 percent by weight of a film-forming binder selected from the group consisting of ethylene-acrylic acid copolymers, polyolefins, and waxes and from about 85 to about 20 percent by weight of a powdered thermoplastic polymer selected from the group consisting of polyolefins, polyesters, polyamides, waxes, epoxy polymers, ethylene-acrylic acid copolymers, and ethylene-vinyl acetate copolymers, wherein each of said film-forming binder and said powdered thermoplastic polymer melts in the range of from about 65° C. to about 180 degrees Celsius and said powdered thermoplastic polymer consists of particles of about 2 to about 50 micrometers in diameter.
9. The kit of claim 1, wherein the transfer material is an image-receptive heat transfer material having front and rear surfaces, which comprises:
- a first layer defining a first surface, and
 - a second layer defining a second surface, which layer comprises particles of a thermoplastic polymer having dimensions of less than about 50 micrometers, from about 10 to about 50 weight percent of a film-forming binder, based on the weight of the thermoplastic polymer, and from about 0.2 to about 10 weight percent of an ink viscosity modifier, based on the weight of the thermoplastic polymer.
10. The kit of claim 1, wherein the transfer material is an image-receptive heat transfer material having front and rear surfaces, which comprises:
- a first layer having first and second surfaces and selected from the group consisting of films and cellulosic nonwoven webs; and

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ing the 80 kDa and 37 kDa proteins for the presence of RNase L proteins.

11. A method of characterizing multiple sclerosis disease activity in a subject, said method comprising:

- (a) identifying the presence of at least one symptom associated with multiple sclerosis in said subject;
- (b) obtaining a PBMC derived sample from said subject;
- (c) determining the ratio of 80 KDa RNase L to 37 KDa RNase L in said sample; and
- (d) using said ratio to characterize MS disease activity in said subject.

12. The method according to claim 11, wherein said determining comprises fractionating said sample into at least two fractions in manner sufficient to separate 80 kDa proteins from 37 kDa proteins.

13. The method according to claim 12, wherein said method further comprises assaying said fractions comprising the 80 kDa and 37 kDa proteins for the presence of RNase L proteins.

14. A kit for use in characterizing multiple sclerosis disease activity in a subject, said kit comprising:

- (a) means for determining the relative amounts of high molecular weight and low molecular RNase L proteins in a subject derived sample; and
- (b) a medium comprising reference information relating relative amounts of high and low molecular weight RNase L proteins to multiple sclerosis.

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15. The kit according to claim 14, wherein said kit further comprises means for obtaining a sample from said subject.

16. The kit according to claim 14, wherein said kit further comprises at least one reagent employed in an oligoclonal CSF assay.

17. A kit for use in characterizing multiple sclerosis disease activity in a subject, said kit comprising:

- (a) means for determining the relative amounts of high molecular weight and low molecular RNase L proteins in a subject derived sample; and
- (b) a medium comprising reference information relating relative amounts of high and low molecular weight RNase L proteins to multiple sclerosis, wherein said kit further comprises instructions for practicing the method of claim 1.

18. A kit for use in characterizing multiple sclerosis disease activity in a subject, said kit comprising:

- (a) means for determining the ratio of high molecular weight and low molecular proteins having RNase L activity in a subject derived sample;
- (b) at least one reagent employed in an oligoclonal CSF assay; and
- (c) instructions for practicing the method of claim 1.

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| COMPONENTS PRESENT IN | WEIGHT PERCENT (WT. %) |
|-------------------------|------------------------|
| tea tree oil | 1 to 10 |
| evening primrose oil | 0.1 to 0.9 |
| aloe barbadensis | 0.1 to 0.9 |
| chamomile extract | 0.1 to 0.9 |
| disodium EDTA | 0.1 to 0.9 |
| citric acid | 0.1 to 0.9 |
| grapefruit seed extract | 0.1 to 0.9 |
| methylparaben | 0.1 to 0.9 |
| propylparaben | 0.01 to 0.09 |
| annatto extract | 0.01 to 0.09 |
| caramel | 0.01 to 0.09 |
| panthenol | 0.1 to 0.9 |
| vitamin E acetate | 0.1 to 0.9, and |
| eucalyptus oil | 0.1 to 0.9. |

and water to remove the therapeutic oil composition and loose cradle cap particles;

(c) repeating step (a) and step (b) as needed for a 1 to 5 day period or until the cradle cap condition disappears.

18. The method of claim 17 to treat cradle cap in a human being, which method comprises:

(a) contacting the scalp or related area of a human being in need of treatment with a therapeutic oil composition itself comprising:

| COMPONENTS PRESENT IN | WEIGHT PERCENT (WT. %) |
|-----------------------|------------------------|
| safflower oil | 60 to 80 |
| sweet almond oil | 5 to 15 |
| tocopheryl acetate | 0.1 to 1 |
| beta - carotene | 0.001 to 0.01 |
| tea tree oil | 1 to 10 |
| propylparaben | 0.01 to 0.09 |
| eucalyptus oil | 0.1 to 1 |
| vitamin A | 0.01 to 0.1, and |
| vitamin B | 0.01 to 0.1. |

rubbing the therapeutic oil composition thoroughly into the area exhibiting cradle cap and allowing the therapeutic oil to remain on the skin surface for between about 1 and 60 min followed by using a brush to gently loosen the scales on the scalp and removing the scales

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(b) contacting the scalp or related area of the human being with an effective amount of the shampoo composition itself comprising:

| COMPONENTS PRESENT IN | WEIGHT PERCENT (WT. %) |
|--------------------------------|------------------------|
| purified soft water | 70 to 88 |
| PEG (80 sorbitan laurate) | 7 to 8 |
| PEG (150 distearate) | 0.3 to 0.3 |
| disodium - lauroamphodiacetate | 8 to 9 |
| sodium lauryl (13 carboxylate) | 3 to 8 |
| cocamidopropyl hydroxysulfate | 3 to 7 |
| decyl glucoside | 4 to 9 |
| tea tree oil | 3 to 8 |
| evening primrose oil | 0.1 to 0.2 |
| aloe barbadensis | 0.1 to 0.5 |
| chamomile extract | 0.1 to 0.4 |
| disodium EDTA | 0.1 to 0.4 |
| citric acid | 0.1 to 0.5 |
| grapefruit seed extract | 0.2 to 0.6 |
| methylparaben | 0.1 to 0.3 |
| propylparaben | 0.005 to .09 |
| annatto extract | 0.01 to 0.06 |
| caramel | 0.04 to 0.09 |
| panthenol | 0.1 to 0.7 |
| vitamin E acetate | 0.1 to 0.5, and |
| eucalyptus oil | 0.1 to 0.5. |

and water to remove the oil and loose cradle cap particles;

(c) repeating step (a) and step (b) as needed for a 1 to 5 day period or until the cradle cap condition disappears.

19. The method of claim 18 wherein the tea tree oil in the shampoo is present in about 8 weight percent.

20. A kit comprising oil, a shampoo composition, a brush, a sponge, a fine toothed comb, and a set of instructions to treat cradle cap according to the method described in claim 17.

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unit and a repeat of the model day study, rhIGF-I/IGFBP-3 treatment decreased 24-hour insulin requirements by 49% (27.3±12.8 vs. 53.6±18.7 U/24 hr; $p<0.001$) and reduced mean home glucose values by 23% (144±73 vs. 187±95 mg/dL; $p<0.02$). All patients receiving rhIGF-I/IGFBP-3 noted a decrease in insulin requirements. The accuracy of the insulin diaries submitted by the patients was confirmed by measuring free insulin in the blood circulation, which was reduced 47% (20.1±9.0 vs. 37.5±15.9) in patients receiving rhIGF-I/IGFBP-3. Growth hormone, known to substantially contribute to insulin resistance, was reduced by 77% in patients receiving rhIGF-I/IGFBP-3 (0.55±0.23 vs. 2.48±0.73).

No significant drug-related side effects were observed. Subjects did not experience edema, jaw pain, or headache-side-effects almost invariably observed when human subjects are treated with substantial doses of free IGF-I. rhIGF-I/IGFBP-3 produced the desirable biological effects of IGF-I in diabetic subjects, but with a surprising lack of side effects.

The patents, patent applications, and publications cited throughout the disclosure are incorporated herein by reference in their entirety.

The present invention has been detailed both by direct description and by example. Equivalents and modifications of the present invention will be apparent to those skilled in the art, and are encompassed within the scope of the invention.

I claim:

1. A method for treatment of diabetes mellitus, comprising:
administering an effective amount of IGF-I/IGFBP-3 complex to a subject suffering from symptoms or complications of diabetes mellitus.
2. The method of claim 1 wherein said IGF-I/IGFBP-3 complex is human IGF-I/IGFBP-3 complex.

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3. The method of claim 2 wherein said IGF-I/IGFBP-3 complex is recombinant human IGF-I/IGFBP-3 complex.

4. The method of claim 1 wherein said diabetes mellitus is type I diabetes mellitus.

5. The method of claim 4 wherein said type I diabetes mellitus is insulin resistant type I diabetes mellitus.

6. The method of claim 1 wherein said diabetes mellitus is type II diabetes mellitus.

7. The method of claim 6 wherein said type II diabetes mellitus is insulin resistant type II diabetes mellitus.

8. The method of claim 1 wherein said diabetes mellitus is type A insulin resistance diabetes mellitus.

9. The method of claim 1 wherein said IGF-I/IGFBP-3 complex is administered on a continuous administration schedule.

10. The method of claim 1 wherein said IGF-I/IGFBP-3 complex is administered on a discontinuous administration schedule comprising cycles which include an on period wherein IGF-I/IGFBP-3 complex is administered and an off period wherein IGF-I/IGFBP-3 complex is not administered.

11. The method of claim 10 wherein said off period is less than said on period.

12. The method of claim 10 wherein said off period is greater than said on period.

13. The method of claim 10 wherein said off period is equal to said on period.

14. A kit, comprising
a package comprising IGF-I/IGFBP-3 complex; and
instructions for use of said IGF-I/IGFBP-3 complex for the treatment of diabetes.

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5. The method of claim 3, wherein the Type V phosphodiesterase is a pyrazolopyrimidinone.

6. The method of claim 5, wherein the Type V phosphodiesterase is sildenafil or a pharmaceutically acceptable salt thereof.

7. The method of claim 1, wherein administration is effected transurethraly.

8. The method of claim 1, wherein administration is effected via intracavernosal injection.

9. The method of claim 1, wherein administration is effected topically.

10. The method of claim 1, wherein administration is effected transdermally.

11. The method of claim 1, wherein the individual is given a daily dose of phosphodiesterase inhibitor in the range of approximately 0.1 to 500 mg/day.

12. The method of claim 1, wherein the phosphodiesterase inhibitor is administered one to four times in a twenty-four hour period.

13. The method of claim 1, wherein the erectile dysfunction is vasculogenic impotence.

14. The method of claim 1, wherein the phosphodiesterase inhibitor is contained within a unit dosage pharmaceutical formulation.

15. The method of claim 11, wherein the pharmaceutical formulation comprises a urethral suppository.

16. The method of claim 15, wherein the urethral suppository contains a pharmaceutically acceptable carrier selected from the group consisting of polyethylene glycol and derivatives thereof.

17. The method of claim 16, wherein the urethral suppository further includes a solubilizing compound for increasing the solubility of the agent in the carrier.

18. A pharmaceutical formulation for treating erectile dysfunction in an individual, comprising a urethral dosage form of a phosphodiesterase inhibitor, a carrier suitable for transurethral drug administration, and, optionally, a transurethral permeation enhancer, wherein the phosphodiesterase inhibitor is a Type V, cGMP-specific phosphodiesterase inhibitor, or a pharmaceutically acceptable salt ester, amide or prodrug thereof.

19. The formulation of claim 18, wherein the urethral dosage form comprises a suppository.

20. A pharmaceutical formulation for treating erectile dysfunction in an individual comprising a sterile liquid composition suitable for intracavernosal administration containing a therapeutically effective amount of a phosphodiesterase inhibitor and a carrier suitable for intracavernosal injection, wherein the phosphodiesterase inhibitor is a Type V, cGMP-specific phosphodiesterase inhibitor, or a pharmaceutically acceptable salt, ester, amide or prodrug thereof.

21. A pharmaceutical formulation for treating erectile dysfunction in an individual, comprising a topical or transdermal composition containing a therapeutically effective amount of a phosphodiesterase inhibitor and a carrier suitable for administration of a drug to the skin or mucosal tissue, wherein the phosphodiesterase inhibitor is a Type V, cGMP-specific phosphodiesterase inhibitor, or a pharmaceutically acceptable salt, ester, amide or prodrug thereof.

22. The formulation of claim 21, wherein the composition is an ointment, cream, gel or lotion.

23. A kit for treating erectile dysfunction in an individual, comprising: a urethral dosage form of a Type V, cGMP-specific phosphodiesterase inhibitor or a pharmaceutically acceptable salt ester, amide or prodrug thereof; a means for transurethrally administering the agent; a container for housing the formulation and drug delivery means; and instruc-

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tions for using the drug delivery means to administer the drug within the context of a dosing regimen effective to treat erectile dysfunction.

24. The kit of claim 23, wherein the means for administering the agent is a transurethral drug delivery device.

25. The kit of claim 23, further including a flexible, adjustable venous flow control (VFC) device and instructions for using the VFC device.

26. The formulation of claim 18, wherein the Type V phosphodiesterase inhibitor is selected from the group consisting of: zaprinast; dipyridamole; pyrazolopyrimidinones; griseolic acid derivatives; 2-phenylpurinones; phenylpyridone derivatives; pyrimidines; pyrimido-pyrimidines; purines; quinazolines; phenylpyrimidinones; imidazoquinoxalinones or aza analogues thereof; phenylpyridones; 4-bromo-5-(pyridylmethylamino)-6-[3-(4-chlorophenyl)propoxy]-3(2H)pyridazinone; 1-[4-[(1,3-benzodioxol-5-ylmethyl)amino]-6-chloro-2-quinazolinyl]-4-piperidine-carboxylic acid, monosodium salt; (+)-cis-5,6a,7,9,9a-hexahydro-2-[4-(trifluoromethyl)-phenylmethyl-5-methylcyclopent-4,5]imidazo[2,1-b]purin-4(3H)one; furazolidin; cis-2-hexyl-5-methyl-3,4,5,6a,7,8,9,9a-octahydrocyclopent[4,5]imidazo-[2,1-b]purin-4-one; 3-acetyl-1-(2-chlorobenzyl)-2-propylindole-6-carboxylate; 4-bromo-5-(3-pyridylmethylamino)-6-(3-(4-chlorophenyl)propoxy)-3(2H)pyridazinone; 1-methyl-5-(5-morpholinoacetyl-2-n-propoxyphenyl)-3-n-propyl-1,6-dihydro-7H-pyrazolo(4,3-d)pyrimidin-7-one; and 1-[4-[(1,3-benzodioxol-5-ylmethyl)amino]-6-chloro-2-quinazolinyl]-4-piperidine-carboxylic acid, monosodium salt.

27. A The formulation of claim 26, wherein the Type V phosphodiesterase inhibitor is zaprinast.

28. The formulation of claim 26, wherein the Type V phosphodiesterase is a pyrazolopyrimidinone.

29. The formulation of claim 28, wherein the Type V phosphodiesterase is sildenafil or a pharmaceutically acceptable salt thereof.

30. The formulation of claim 18, further including an additional active agent.

31. The formulation of claim 30, wherein the additional active agent is a nitric oxide donor.

32. The formulation of claim 31, wherein the nitric oxide donor is selected from the group consisting of nitroglycerin, isosorbide dinitrate, erythrityl tetranitrate, amyl nitrate, sodium nitroprusside, molsidomine, linsidomine chlorhydrate, S-nitroso-N-acetyl-d,l-penicillamine, S-nitroso-N-cysteine, S-nitroso-N-glutathione, diazenium diolates, and combinations thereof.

33. The formulation of claim 30, wherein the additional active agent is a prostaglandin or a pharmaceutically acceptable salt or ester thereof.

34. The formulation of claim 33, wherein the additional active agent is a naturally occurring prostaglandin or a pharmaceutically acceptable ester thereof.

35. The formulation of claim 34, wherein the naturally occurring prostaglandin is selected from the group consisting of PGE₀, PGE₁, PGA₁, PGB₁, PGF_{1α}, 19-hydroxy-PGA₁, 19-hydroxy-PGB₁, PGE₂, PGA₂, PGB₂, 19-hydroxy-PGA₂, 19-hydroxy-PGB₂, PGE₃ and PGF_{3α}.

36. The formulation of claim 33, wherein the additional active agent is a synthetic prostaglandin.

37. The formulation of claim 36, wherein the synthetic prostaglandin is selected from the group consisting of carboprost tromethamine, dinoprost tromethamine, dinoprostone, lipoprost, gemeprost, metenoprost, sulprostone, tiaprost and combinations thereof.

38. The formulation of claim 30, wherein the additional active agent is an α-blocker selected from the group con-

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sion to form a binding reaction admixture in the syringe barrel. The reaction admixture was then maintained as before, and then the solution in the syringe was expelled to separate that solution from the retained insoluble DNA-matrix complex. In a similar manner, washes and elution were performed by drawing up the respective wash and elution buffers as described in Example 4. The eluted sample was collected as it was expelled from the outlet of the syringe-mounted filter. When this eluted sample was analyzed on agarose gels for plasmid DNA it was found to be comparable in yield and purity to the isolated DNA described in Example 4.

6. Particulate Glass Isolation of DNA Using a Sedimentation Based Separation Step

The above methods for isolating DNA may be modified to accommodate wash and elution steps based on sedimentation at unit gravity as a means for separation in place of centrifugation. This modified procedure is conducted in the same manner as described in Example 2 or Example 4 except that the described centrifugation steps are substituted with the following sedimentation-based separation steps as noted.

The dissolved pellet prepared in Example 4 is added to a well of a microtiter plate and admixed therein with the binding buffer and the suspension of particulate glass as in Example 4 to form a binding reaction admixture.

The fraction of particulate glass used for a sedimentation-based separation is a fraction prepared as in Example 1 except that it is fractionated such that more than 98% of the glass particles in the fraction sediment under unit gravity over a 1 cm vertical distance in a time period between 15 seconds and 2 hours. For the purpose of saving time between washes, it is preferred if the sedimentation time occurs between 2 and 6 minutes.

The reaction admixture in the microtiter wells is maintained as in Example 4. Thereafter, in place of centrifugation the plates are held stationary and the particulate glass is allowed to settle. The liquid layer present above the settled glass is decanted (separated) and wash buffer is then admixed with the settled glass present in the microtiter well. Settling, separating and further admixing steps are then performed in this sedimentation-based mode to subject the glass particles to washes and elution essentially as described in Example 4 thus carried out to conduct the to yield isolated DNA in the first separated elutant solution.

7. Particulate Glass Isolation of DNA Using a Centrifugation Based Separation Step

The above methods for isolating nucleic acids was modified to accommodate a procedure having simplified binding, wash and elution steps. In the present modified procedure, a centrifugal filtration step was used to separate the particulate glass from the various buffers in place of a centrifugation/decanting step, or the simple pressure filtration step described in Example 5. This modified procedure was generally the same as in Example 2 or Example 4 with the following exceptions to the steps for separating the particulate glass from any of the various buffers as noted.

As before, a solution containing nucleic acid is admixed with binding buffer and the particulate glass to form a binding reaction admixture, and the admixture was maintained as before to allow the formation of an insoluble DNA-matrix complex. Thereafter, the admixture was placed

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into the upper chamber of a SPIN-X centrifugal filtration unit (COSTAR, Cambridge, Mass.) and centrifuged on a microfuge apparatus for 15 seconds at about 12,000xg to force the liquid phase of the admixture through the filter into the lower chamber of the unit. The particulate glass bound nucleic acid molecules remain in the upper chamber of the filter unit. Thereafter, the particulate glass is washed three times with binding buffer and three times with wash buffer as described before, except at each wash, the liquid is removed by a centrifugation step to pass the liquid through the filter. The lower chamber was emptied as needed to allow for the repeated washes. Finally, the bound nucleic acids were eluted by the addition of elution buffer to the particulate glass as described before, except that the elution buffer was added to the upper chamber, and the unit was centrifuged to pass the elution buffer and eluted nucleic acids into the lower chamber where it was collected, thereby separating the nucleic acid from the particulate glass.

The use of the centrifugal filtration procedure yielded nucleic acid of similar purity as described before by the present methods. However, the resulting nucleic acids, when high molecular weight DNA was isolated by the present method, provides material that is less sheared (broken) than DNA isolated by multiple binding, washing and eluting steps that require resuspension and pipetting manipulations. Thus, the centrifugal filtration method yields superior high molecular weight DNA.

The foregoing specification, including the specific embodiments and examples, is intended to be illustrative of the present invention and is not to be taken as limiting. Numerous other variations and modifications can be effected without departing from the true spirit and scope of the present invention.

What is claimed is:

1. A composition for isolating nucleic acid molecules consisting essentially of particulate glass having a sedimentation time through 100 centimeters (cm) of still water at unit gravity in the range of 6 weeks to 20 minutes.
2. The composition of claim 1 wherein said sedimentation time is in the range of 6 weeks to 2 hours.
3. The composition of claim 1 wherein said sedimentation time is in the range of 1 week to 2 hours.
4. The composition of claim 1 wherein said sedimentation time is in the range of 6 weeks to 1 week.
5. A kit for isolating nucleic acid molecules comprising a composition consisting essentially of particulate glass having a sedimentation time through 100 centimeters (cm) of still water at unit gravity in the range of 6 weeks to 20 minutes, said kit further containing instructions for use of said composition for isolating said nucleic acid molecules.
6. The kit of claim 5 wherein said sedimentation time is in the range of 6 weeks to 2 hours.
7. The kit of claim 5 wherein said sedimentation time is in the range of 1 week to 2 hours.
8. The kit of claim 5 wherein said sedimentation time is in the range of 6 weeks to 1 week.
9. The kit of claim 5 further containing a filtration means having a pore size that retains the particulate glass and passes solutions.
10. The kit of claim 9 wherein said pore size is 0.1 to 1.0 micrometers.
11. The kit of claim 9 wherein said filtration means is a filter in a pressurizable chamber having an inlet before the filter for delivery of liquid and an outlet after the filter for collecting liquid that passes the filter.
12. The kit of claim 9 wherein said filtration means is a filter in a centrifuge tube having an upper chamber above the

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(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

| | |
|---|-----|
| TCAGATCTAC AGAAGGCTGT TCTTACATGA AGAAGGCTGT GAAGGCTGAA CAATCATGGA | 60 |
| TTTTTCTGAT CAATTGTGCT TTACGAAATT ATTGACAGTT TTGCACAGGT TCTTGAAAAC | 120 |
| GTTATTATA ATGAAATCAA CTAAGACTAT TTTTGCTATA AGTTCATATA GGTGCATAAA | 180 |
| ACCCTTAAT TCATCTACTA GCTGTTCCCC CGAACAGGTT TATTTIAGTA AAAAAAAAAA | 240 |
| AGCAAAAAAC AAAACAAAA GATTTTATC AAATGTTATG ATGCAAAAAA AAAAAA | 296 |

What is claimed is:

1. A method of identifying a biological indicator of exposure to ionizing radiation, comprising the steps of: exposing a population of cells to ionizing radiation; using differential display to compare gene expression in the population of cells exposed to the ionizing radiation to gene expression in a control population of cells not exposed to the ionizing radiation; and selecting a serum amyloid gene or serum amyloid gene fragment that has an altered level of gene expression in the exposed population of cells as compared to the control population of cells, which level of expression persists for at least three weeks following exposure to the ionizing radiation.
2. A method according to claim 1, additionally comprising a step of sequencing the gene or gene fragment.
3. A method according to claim 1, wherein the step of exposing a population of cells to ionizing radiation is a step of exposing a culture of cells in vitro.
4. A method according to claim 1, wherein the step of exposing a population of cells to ionizing radiation is a step of exposing cells in vivo.
5. A method according to claim 1, wherein the serum amyloid is murine serum amyloid A3.
6. A method according to claim 1, wherein the serum amyloid is human serum amyloid A1 or A2.
7. A method according to claim 1, wherein the cells are bone marrow cells.
8. A method according to claim 1, wherein the gene or gene fragment has a level of gene expression in the exposed population of cells that is increased as compared to the control population of cells.
9. A method according to claim 8, wherein the increased level of expression is at least double that found in the control population.
10. A method according to claim 8, wherein the level of the biological indicator in the control population is virtually undetectable by conventional techniques.
11. A method of determining whether an individual has been exposed to radiation, comprising the steps of obtaining cells from an individual and then assaying the cells for the presence of a gene or gene fragment that corresponds to a gene that encodes a serum amyloid that persists for at least three weeks following exposure to radiation.
12. A method according to claim 11, additionally comprising a step of culturing the cells prior to the step of assaying the cells.
13. A method according claim 11, wherein Northern blot analysis is used in the assaying step.

14. A method according claim 11, wherein RT-PCR analysis is used in the assaying step.

15. A method according to claim 11, wherein the biological marker is a gene or gene fragment corresponds to a gene that encodes a serum amyloid.

16. A method according to claim 11, wherein the serum amyloid is murine serum amyloid A3.

17. A method according to claim 11, wherein the serum amyloid is human serum amyloid A1 or A2.

18. A method according to claim 11, wherein the cells are bone marrow cells.

19. A method according to claim 11, wherein the gene or gene fragment has a level of gene expression in the exposed population of cells that is increased as compared to the control population of cells.

20. A method according to claim 19, wherein the increased level of expression is at least double that found in the control population.

21. A method according to claim 19, wherein the level of the biological indicator in the control population is virtually undetectable by conventional techniques.

22. A kit for detecting past exposure to ionizing radiation, comprising:

primers specific for a serum amyloid gene or serum amyloid gene fragment that changes its level of expression in response to exposure to ionizing radiation, which level of expression persists for at least three weeks following the exposure to the ionizing radiation; reagents for RT-PCR analysis; and

instructions for using the primers and the reagents in an assay to determine whether cells removed from a subject have an altered level of expression of the gene or gene fragment as compared to expression in cells in a control population.

23. A kit as claimed in claim 22, wherein the cells are bone marrow cells.

24. A kit as claimed in claim 23, wherein the primers are specific to a serum amyloid A gene or fragment thereof.

25. A method according to claim 11, wherein the biological indicator is one that persists for at least three months following exposure to radiation.

26. A method according to claim 11, wherein the biological indicator is one that persists for at least one year following exposure to radiation.

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drate is preferably a combination of glucose and a less rapidly absorbed carbohydrate of low-to-medium glycemic index. The concentrate is dissolved in 375 ml of cold water and consumed 15 minutes before exercise.

Post-exercise: A concentrate comprising 100 grams of glucose and 1160 mg of sodium chloride. The concentrate is dissolved in 500 ml of water and is consumed shortly after exercise.

Example III

The regimen of this example is intended for use with intermittent, high-intensity, repetitive exercise of duration less than 1 hour. It consists entirely of fully-constituted liquid formulations. It accomplishes pre-exercise loading of alkaline salt (sodium bicarbonate) to counter the effects of lactic acid production on high-intensity muscle performance, and post-exercise loading of carbohydrate and creatine to augment ATP resynthesis and to augment muscle glycogen stores. The pre-exercise formulations anticipate fluid needs. Carbohydrate is loaded just prior to exercise to avoid insulin surge. Conversely, short acting carbohydrate is loaded after exercise to stimulate insulin output which augments creatine uptake by muscles. In view of pre-exercise sodium loading, sodium is not incorporated in formulations for use after exercise. Preferably, fructose is avoided in this regimen since both bicarbonate and fructose may be associated with stomach upset in some individuals.

Pre-exercise: 20 grams of sodium bicarbonate and 45 grams of glucose in 500 ml of water, 250 ml to be consumed 45 minutes prior to exercise and 250 ml to be consumed 15 minutes prior to exercise.

Post-exercise: 100 grams of glucose and 8 grams creatine monohydrate in 500 ml of water to be consumed shortly after exercise.

Thus it has been shown and described an assemblage of nutrient beverage formulations for use before and after exercise for enhancing performance during exercise and reducing adverse effects of exercise which satisfies the objects set forth above.

Since certain changes may be made in the present disclosure without departing from the scope of the present invention, it is intended that all matter described in the foregoing specification and shown in the accompanying drawings be interpreted as illustrative and not in a limiting sense.

What is claimed is:

1. An exercise beverage kit comprising:

- (a) a first beverage having a volume of water of from about 300 milliliters to about 600 milliliters and a concentration of carbohydrate of about 9% or less;
- (b) a second beverage having a volume of water of from about 300 milliliters to about 600 milliliters and a concentration of carbohydrate of greater than about 16%;
- (c) indicia to distinguish said first and second beverages;

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(d) instructions which teach the use of said first and second beverages as a regimen in association with exercise such that said first beverage is instructed for ingestion prior to exercise having a duration of about one hour or less, and said second beverage is instructed for ingestion directly following said exercise; and

(e) packaging unifying said first and said second beverages, indicia and, instructions.

2. The kit of claim 1 wherein said carbohydrate of said second beverage has a high glycemic index.

3. The kit of claim 1 wherein only said first beverage contains caffeine.

4. A method for preparing an exercise hydration and muscle glycogen replenishment kit comprising the steps of:

(a) formulating a first beverage to have a volume of water of from about 300 milliliters to about 600 milliliters and a concentration of carbohydrate of less than about 9%;

(b) formulating a second beverage to have a volume of water of from about 300 milliliters to about 600 milliliters and a concentration of carbohydrate of greater than about 20%;

(c) devising indicia to distinguish said first and second beverages;

(d) devising instructions to teach the use of said first and second beverages as a regimen in association with exercise such that said first beverage is instructed for ingestion prior to exercise having a duration of about one hour or less, and said second beverage is instructed for ingestion directly following said exercise; and

(e) providing said first and second beverages, indicia and, instructions in a unified package for a user.

5. A method for exercise hydration and muscle glycogen replenishment comprising the steps of:

(a) obtaining an exercise beverage kit comprising a first beverage having a volume of water of from about 300 milliliters to about 600 milliliters and a concentration of carbohydrate of less than about 9%, a second beverage having a volume of water of from about 300 milliliters to about 600 milliliters and a concentration of carbohydrate of greater than about 20%, indicia to distinguish said first and second beverages, instructions which teach the use of said first and second beverages as a regimen in association with exercise having a duration of about one hour or less, and packaging unifying said first and second beverages, indicia, and instructions;

(b) using said indicia to distinguish said beverages;

(c) drinking said first beverage prior to said exercise, in accordance with said instructions; and

(d) drinking said second beverage directly following said exercise, in accordance with said instructions.

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3. The method of claim 1, wherein the step of scrubbing with a non-oil-based cleanser comprises scrubbing with an anti-bacterial soap.

4. The method of claim 3, wherein the step of scrubbing with an anti-bacterial soap comprises providing anti-bacterial soap having an active ingredient including triclosan.

5. The method of claim 3, wherein the step of scrubbing with an anti-bacterial soap comprises scrubbing in a circular motion for at least two minutes, including scrubbing a plurality of infected areas of the skin surface to be shaved.

6. The method of claim 1, wherein the step of preparing the skin surface comprises avoiding use of oil, oil-based, and lanolin products on the skin surface to be shaved.

7. The method of claim 1, wherein the step of shaving comprises the steps of:

drawing the skin surface tight to create a smooth surface to which the razor is to be applied; and

pressing lightly enough with the razor to avoid creating an indentation in the skin surface.

8. The method of claim 1, wherein the step of shaving comprises applying a lather with a barber brush.

9. The method of claim 8, wherein the step of applying a lather comprises applying a lather from an anti-bacterial soap in a circular motion with a barber's brush including boar's hair.

10. The method of claim 1, wherein the step of pulling the razor comprises pulling the razor downward in a plurality of single strokes on both sides of a face.

11. The method of claim 10, wherein the step of pulling the razor further comprises the steps of:

tilting a head back to tighten a portion of skin at a neck; and

pulling the razor gently upward from a low point on the neck to a point where pulling the razor downward was stopped.

12. The method of claim 10, wherein the step of pulling the razor further comprises:

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pulling the razor from one of the sides of the face toward a center of a chin while placing a tongue in a gum line to tighten a portion of skin below a bottom lip; and

pulling the razor from another of the sides of the face toward the center of the chin while placing the tongue in the gum line.

13. The method of claim 12, wherein the step of pulling the razor further comprises pulling the razor downward from the lower lip toward the chin while placing the tongue in the gum line.

14. A method of reducing ingrown hairs comprising the steps of:

preparing a skin surface to be shaved for four days prior to shaving by scrubbing the skin surface twice-a-day with an anti-bacterial soap for at least two minutes;

avoiding application of any oil-based products to the skin surface during the preparing step; and

shaving the skin surface on a fifth day with a plurality of uni-directional motions without shaving a plurality of hairs below the skin surface.

15. A kit for reducing folliculitis comprising:

a razor;

an anti-bacterial soap; and

a set of instructions delineating a process for reducing folliculitis.

16. The kit of claim 15, further comprising a barber's brush.

17. The kit of claim 16, wherein the barber's brush comprises boar's hair.

18. The kit of claim 15, wherein the anti-bacterial soap has an active ingredient including triclosan.

19. The kit of claim 15, further comprising:

a boar's hair barber's brush; and

wherein the anti-bacterial soap has an active ingredient including triclosan.

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Benzoylmethyl Disulfide, Formamidine Disulfide 2HCl, 2-(Diethylamino)ethyl Disulfide, Aldrithiol-2, Aldrithiol-4, 2,2-Dithiobis(Pyridine N-Oxide), 6,6-Dithiodinicotinic Acid, 4-Methyl-2-Quinolyl Disulfide, 2-Quinolyl Disulfide, 2,2-Dithiobis(benzothiazole), 2,2-Dithiobis(4-Tert-Butyl-1-Isopropyl)-Imidazole, 4-(dimethylamino)phenyl disulfide, 2-Acetamidophenyl Disulfide, 2,3-Dimethoxyphenyl Disulfide, 4-Acetamidophenyl Disulfide, 2-(Ethoxycarboxamido)phenyl Disulfide, 3-Nitrophenyl Disulfide, 4-Nitrophenyl Disulfide, 2-Aminophenyl Disulfide, 2,2-Dithiobis(benzonitrile), p-Tolyl Disulfoxide, 2,4,5-Trichlorophenyl Disulfide, 4-Methylsulfonyl-2-Nitrophenyl Disulfide, 4-Methylsulfonyl-2-Nitrophenyl Disulfide, 3,3-Dithiodipropionic Acid, N,N-Diformyl-L-Cystine, Trans-1,2-Dithiane-4,5-Diol, 2-Chloro-5-Nitrophenyl Disulfide, 2-Amino-4-Chlorophenyl Disulfide, 5,5-Dithiobis(2-Nitrobenzoic Acid), 2,2-Dithiobis(1-Naphthylamine), 2,4-Dinitrophenyl p-Tolyl Disulfide, 4-Nitrophenyl p-Tolyl Disulfide, and 4-Chloro-3-Nitrophenyl Disulfideformamidine disulfide dihydrochloride.

8. The method of claim 6, wherein said retrovirus is selected from the group consisting of Lentiviruses and Oncoviruses.

9. The method of claim 6, wherein said retrovirus is a HIV-1 retrovirus.

10. The method of claim 6, wherein the method further comprises contacting said retrovirus with an anti-retroviral agent.

11. The method of claim 6, wherein the method further comprises contacting said retrovirus with a nucleotide analogue.

12. The method of claim 6, wherein the method further comprises contacting said retrovirus with AZT.

13. A method of selecting a compound capable of dissociating a zinc ion chelated with a CCHC zinc finger of a retroviral nucleocapsid protein, said method comprising:

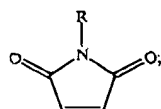
(a) contacting the CCHC zinc finger of said retroviral nucleocapsid protein with an electron acceptor; and

(b) detecting the dissociation of said zinc ion from the CCHC zinc finger of said retroviral nucleocapsid protein,

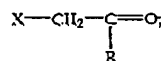
wherein said electron acceptor is a compound selected from the group consisting of

disulfides having the formula $R-S-S-R$;

maleimides having the formula



α -halogenated ketones having the formula



hydrazides having the formula $R-NH-NH-R$;
nitric oxide and derivatives containing the NO group;
cupric ions and complexes containing Cu^{+2} ; and
ferric ions and complexes containing Fe^{+3} ;
wherein the compound is not a C-nitroso compound of the formula $R-C-NO$, and wherein R is any atom or molecule, and X is selected from the group consisting of F, I, Br and Cl.

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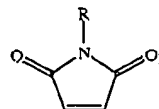
14. The method of claim 13, wherein said electron acceptor is a compound selected from the group consisting of Tetramethylthiuram Disulfide, Tetractylthiuram Disulfide, Tetraisopropylthiuram Disulfide, Tetrabutylthiuram Disulfide, Dicyclopentamethylenethiuram Disulfide, Isopropylxanthic Disulfide, O,O-Diethyl Dithiobis-(Tbioformate), Benzoyl Disulfide, Benzoylmethyl Disulfide, Formamidine Disulfide 2HCl, 2-(Diethylamino)ethyl Disulfide, Aldrithiol-2, Aldrithiol-4, 2,2-Dithiobis(Pyridine N-Oxide), 6,6-Dithiodinicotinic Acid, 4-Methyl-2-Quinolyl Disulfide, 2-Quinolyl Disulfide, 2,2-Dithiobis(benzothiazole), 2,2-Dithiobis(4-Tert-Butyl-1-Isopropyl)-Imidazole, 4-(dimethylamino)phenyl disulfide, 2-Acetamidophenyl Disulfide, 2,3-Dimethoxyphenyl Disulfide, 4-Acetamidophenyl Disulfide, 2-(Ethoxycarboxamido)phenyl Disulfide, 3-Nitrophenyl Disulfide, 4-Nitrophenyl Disulfide, 2-Aminophenyl Disulfide, 2,2-Dithiobis(benzonitrile), p-Tolyl Disulfoxide, 2,4,5-Trichlorophenyl Disulfide, 4-Methylsulfonyl-2-Nitrophenyl Disulfide, 4-Methylsulfonyl-2-Nitrophenyl Disulfide, 3,3-Dithiodipropionic Acid, N,N-Diformyl-L-Cystine, Trans-1,2-Dithiane-4,5-Diol, 2-Chloro-5-Nitrophenyl Disulfide, 2-Amino-4-Chlorophenyl Disulfide, 5,5-Dithiobis(2-Nitrobenzoic Acid), 2,2-Dithiobis(1-Naphthylamine), 2,4-Dinitrophenyl p-Tolyl Disulfide, 4-Nitrophenyl p-Tolyl Disulfide, and 4-Chloro-3-Nitrophenyl Disulfideformamidine disulfide dihydrochloride.

15. The method of claim 13 wherein said step of detecting the dissociation of said zinc ion from the CCHC zinc finger of said retroviral nucleocapsid protein is carried out using a method selected from the group consisting of capillary electrophoresis, immuno-blotting, Nuclear Magnetic Resonance (NMR), high pressure liquid chromatography (HPLC), detecting release of radioactive zinc-65, detecting fluorescence, and detecting gel mobility shift.

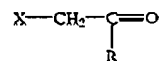
16. A kit for selecting a compound capable of dissociating a zinc ion from a CCHC zinc finger of a nucleocapsid protein, said kit comprising a retroviral nucleocapsid protein and instructions for detecting the dissociation of said zinc ion from said nucleocapsid protein, the instructions comprising directions for the selection of a compound selected from the group consisting of:

disulfides having the formula $R-S-S-R$;

maleimides having the formula



α -halogenated ketones with the structure



hydrazides having the formula $R-NH-NH-R$;
nitric oxide and derivatives containing the NO group;
cupric ions and complexes containing Cu^{+2} ; and
ferric ions and complexes containing Fe^{+3} ;
wherein the compound is not a C-nitroso compound of the formula $R-C-NO$, and wherein R is any atom or molecule, and X is selected from the group consisting of F, I, Br and Cl.

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What is claimed is:

1. A method for percutaneously accessing an implanted port in a patient, said method comprising:

- (a) providing a needle;
- (b) aligning the needle with an aperture on the port, wherein the port is subcutaneously connected to a blood vessel and contains a valve which isolates the aperture from the blood vessel;
- (c) percutaneously introducing the needle through tissue overlying the port and into the aperture, wherein the needle opens a blood flow path between the blood vessel and the port;
- (d) flowing blood between the blood vessel and a catheter attached to the needle through the port; and
- (e) withdrawing the needle from the aperture, wherein the valve closes to inhibit bleeding from the blood vessel to a tissue tract created by the needle;
- (f) providing another needle;
- (g) aligning the other needle with the aperture on the port;
- (h) percutaneously introducing the other needle through the same tissue tract into the aperture, wherein the needle opens a blood flow path between the blood vessel and the port;
- (i) flowing blood between the blood vessel and a catheter attached to the other needle through the port; and

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(j) withdrawing the other needle from the aperture, wherein the valve closes to inhibit bleeding from the blood vessel to a tissue tract created by the needle.

2. A method as in claim 1, wherein the needle has a bore size of at least 1.16 mm.

3. A method as in claim 1, wherein the other needle has a bore size of at least 1.16 mm.

4. A method as in claim 1, wherein the steps (g) through (j) are performed from two hours to four days after step (e).

5. A method as in claim 1, further comprising repeating steps (f) through (j) periodically.

6. A method as in claim 5, wherein the steps (f) through (j) are repeated every two hours to every four days.

7. A method as in claim 1, wherein the needles are introduced in a direction generally normal to the skin surface at the point through which it passes.

8. A kit comprising:

a needle;

instructions for use setting forth the method of claim 1; and

a package containing the port and instructions.

9. A kit as in claim 8, further comprising a catheter connected or connectable to the needle.

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animals a methanogen growth inhibiting amount of an HMG-CoA reductase inhibitor.

12. The method of claim 11 wherein said HMG-CoA reductase inhibitor produces inhibition of growth of a methanogenic bacterial species at a concentration which produces less than 30% inhibition of *Ruminococcus albus*.

13. The method of claim 11 wherein said ruminants are cattle, sheep or goats.

14. The method of claim 11 wherein the HMG-CoA reductase inhibitor is chosen from the group consisting of atorvastatin, fluvastatin, lovastatin, mevastatin, pravastatin, and simvastatin.

15. The method of claim 11 wherein the HMG-CoA reductase inhibitor is administered to animals in admixture with an ordinary solid foodstuff, in feeding water or, for young animals, dissolved or suspended in whole milk or skim milk, or in the form of a slow-release, intra-ruminal pellet or bolus.

16. The method of claim 11 wherein said methanogen growth inhibiting amount of HMG-CoA reductase inhibitor

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is sufficient to inhibit methanogens but insufficient to inhibit non-methanogenic fermentative bacteria in said ruminant animal.

17. A ruminant feed or feed concentrate comprising:

- (a) a standard ruminant feed, and
- (b) a methanogen growth inhibiting amount of an HMG-CoA reductase inhibitor.

18. The feed of claim 17 wherein said HMG-CoA reductase inhibitor is chosen from the group consisting of atorvastatin, fluvastatin, lovastatin, mevastatin, pravastatin, and simvastatin.

19. A kit for preparing a ruminant feed comprising:

- (a) an HMG-CoA reductase inhibitor; and
- (b) instructions for mixing said HMG-CoA reductase inhibitor with a standard ruminant feed.

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